THE SORPTION OF DISSOLVED HEAVY METALS FROM STORMWATER: THE EFFECT OF BIOFILM, GRAVEL, AND TWO ALTERNATIVE SUBSTRATES.

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Abstract

Stormwater acts as a major transport pathway for urban contaminants, with heavy metal contaminants of concern primarily being zinc, copper, and lead. In an effort to reduce the environmental impacts of stormwater, a range of stormwater treatment systems have been developed, collectively called Low Impact Developments (LIDs). Environmentally they aid in reducing contaminant loads entering surfacewater and groundwater systems while enhancing the aesthetic values of urban areas. Most current stormwater treatment systems incorporate both filtration and infiltration processes in contaminant removal restricting their remedial properties to particulate contaminants. This can be limiting if much of the contaminants are in dissolved form.

Research into alternative stormwater treatment systems substrate materials (e.g. recycled glass and mussel shell) for stormwater treatment is necessary to provide sustainable and environmentally friendly options for the potential treatment of dissolved heavy metals. In addition to alternative materials, the effect of biofilm on the adsorption of dissolved heavy metals needs to be studied. Biofilms are assemblages of microbial cells connected with extracellular polymeric substances (EPS) that attach themselves to moist abiotic surfaces. Biofilms are known to sorb heavy metals from aqueous environments, in particular, dissolved heavy metals which have the potential to escape treatment in stormwater treatment systems. Thus, there is a need to understand the role of alternative materials and biofilm in the sorption of dissolved heavy metals from stormwater, in an effort to improve the efficiency of stormwater treatment systems.

This thesis investigated the use of recycled glass, mussel shell, gravel, and biofilm for the treatment of dissolved heavy metals in a stormwater. Freshwater biofilm utilised throughout this research was grown using water collected from Okeover and Haytons Streams, as well as, seed biofilm from Haytons Stream and activated sludge from the Bromley Sewage Ponds in Christchurch, New Zealand. A synthetic stormwater, containing typical concentrations of heavy metal contaminants, was used for all experiments.

The biofilm demonstrated an ability to adsorb dissolved zinc (Zn), copper (Cu), and lead (Pb) to high concentrations from the stormwater. However, the biofilm did not have a significant impact on heavy metal removal relative to the substrate on its own. In fact, the results would suggest that the presence of biofilm inhibits the removal of certain dissolved heavy metals in either or both the recycled glass and mussel shell substrates.

The gravel, recycled glass, and mussel shell substrates demonstrated stormwater treatment potential by effectively reducing the concentration of dissolved heavy metals from the synthetic stormwater. Gravel showed a removal efficiency (%) of Pb (99%) ≥ Zn (99%) > Cu (96%) and mussel shell one of Pb (97%) ≥ Zn (97%) > Cu (89%). Meanwhile, recycled glass achieved an order of removal efficiency

of Pb (96%) > Zn (86%) > Cu (73%). Dissolved heavy metal removal mechanisms were investigated using scanning electron microscope and energy-dispersive X-ray spectroscopy analyses and it is likely that both the composition and surface texture of the substrate influence the removal of dissolved heavy metal from aqueous solutions.

The results of this thesis demonstrated benefits in the use of waste products for the protection of the environment. Using recycled glass and mussel shells for stormwater treatment contributes to waste minimisation and increased value associated with the extended lifecycle of these products.

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Terminology

Abbreviations and Acronyms

ANZECC	Australian and New Zealand Environmental and Conservation Council
APAH	American Public Health Association
BOD	Biological Oxygen Demand
BMP	Best management practice
$CaCO_3$	Calcium carbonate
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
Cu	Copper
DI	Deionized water
DOC	Dissolved Organic Carbon
EDS	Energy-dispersive x-ray spectroscopy
EPS	Extracellular polymeric substances
HCL	Hydrochloric acid
HCO_3-	Bicarbonate
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
KCL	Potassium chloride
LID	Low impact development
MTBE	Methyl tert-butyl ether
n	Number of samples
N	Nitrogen
NO_3-N	Nitrate
NH_4-N	Ammonium nitrate
PAH	Polycyclic aromatic hydrocarbon
Pb	Lead
PCB	Polychlorinated biphenyls
PMP	Polymethylpentene
RMA	Resource Management Act 1991
SEM	Scanning electron microscope
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
Zn	Zinc

Units

g	grams
hr	hour
kg	kilogram
L	litre
m	metre
mg	milligram
mL	millilitre
mm	millimetre
mol	mole
V	volts
µg	microgram
µm	micrometre
µS/cm	microsiemen
Wt%	percentage weight

Chapter 1. Introduction

1.1 Overview

Throughout the world, urbanisation is increasing and as a result, the area of impervious surfaces is growing with it. These changes ultimately lead to changes in surface runoff characteristics within urban catchments, resulting in higher discharge peaks and runoff volumes. Stormwater is the result of runoff produced on impervious surfaces during rainfall events and acts as a major transport pathway for urban contaminants that accumulate on impervious surfaces. Common contaminants include sediments, organic micropollutants (most commonly polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)), pathogenic microorganisms, nutrients, and heavy metals (Barbosa et al., 2012). These urban contaminants can act as non-point source pollutants as they often bypass natural filtration processes provided by soil and vegetation before they enter waterways. As a result, the ecological, recreational, and aesthetic values of the receiving waterways are compromised.

In an effort to reduce the environmental impacts of stormwater, a range of stormwater treatment systems have been developed, collectively called Low Impact Developments (LIDs). These systems include retention basins, wetlands, rain gardens (bioretention systems), green roofs, and swales, and ultimately work to reduce the quantity and slow the flow of runoff from impervious surfaces. Environmentally they aid in reducing contaminant loads entering surfacewater and groundwater systems while enhancing the aesthetic values of urban developments and providing habitats for wildlife (Charlesworth et al., 2003). Stormwater treatment systems incorporate both filtration and infiltration processes in contaminant removal. However, their effectiveness can be restricted to particulate contaminants and if the majority of contaminants are in dissolved form, the remedial properties of stormwater treatment systems can be limited. Biofilms are assemblages of microbial cells connected with extracellular polymeric substances (EPS) that attach themselves to moist abiotic surfaces (Singh et al., 2006). Found throughout the environment, biofilms may work to improve the bioremedial properties of stormwater treatment systems, in particular, by the active and passive uptake of heavy metals from the surrounding environment.

Research has demonstrated the ability of biofilms to sorb heavy metals from aqueous environments (e.g. Ancion et al., 2010; Feder et al., 2015; Hansda et al., 2016) and current understanding would suggest that the presence of biofilms in stormwater treatment systems might improve the removal of heavy metals from stormwater. In particular, dissolved heavy metals, which have the potential to escape treatment, and be discharged into receiving waterways. Thus, there is a need to understand the role of biofilms in the sorption of dissolved heavy metals from stormwater in an effort to improve the efficiency of stormwater treatment systems by enhancing dissolved metal uptake to prevent environmental degradation. Furthermore, research into alternative materials for stormwater treatment is increasing as

the need for more sustainable and environmentally friendly options becomes necessary. This research investigated the use of mussel shell and recycled glass, both with and without biofilm, as alternative substrates for the treatment of dissolved heavy metals in stormwater, as well as gravel, which is a substrate commonly used in stormwater treatment system construction currently.

1.2 Research Aims and Objectives

The aim of this research is to assess whether the growth of biofilms on substrate can improve the removal of dissolved heavy metals from stormwater treatment systems, and to evaluate the potential use of recycled glass, mussel shell, and gravel for dissolved heavy metal treatment.

The main objectives of this research are:

1. To quantify how effectively biofilms sorb dissolved heavy metals from stormwater over time;
2. To determine if biofilms adsorb dissolved heavy metals from a synthetic stormwater to a greater extent than substrate alone;
3. To determine the efficiency of recycled glass, mussel shell, and gravel substrates to remove dissolved heavy metals from a synthetic stormwater;
4. To investigate the dissolved heavy metal removal mechanism(s) of the recycled glass, mussel shell, and gravel substrates.

1.3 Thesis Structure

The structure of this thesis includes an introduction (Chapter 1) which briefly presents the basis of the thesis as well as context and background knowledge. The literature review (Chapter 2) introduces key concepts and expands on background knowledge relevant to the basis of this thesis. The methods chapter (Chapter 3) defines the approach, rational, tools, and quality assurance utilised in this research to test the objectives outlined in the introduction. The outcomes obtained from the experiments are presented in the results section (Chapter 4) and then discussed in Chapter 5, followed by conclusions and future research recommendations (Chapter 6).

Chapter 2. Literature Review

2.1 Stormwater

Land use modifications to the natural environment, particularly urbanisation, regularly result in changes to the surface runoff hydrograph of the catchment. Urban infrastructure and its associated land use modifications, such as vegetation removal, drainage channel modifications, and the replacement of pervious surfaces with impervious surfaces, results in increased water runoff during periods of precipitation. The movement of water over impervious surfaces during precipitation is termed stormwater (Barbosa et al., 2012), and as the area of imperviousness increases, there are two major impacts on stormwater: changes in water quantity (hydrology), and increased water quality impairment. Despite these affects, stormwater can exhibit distinct characteristics that can be site specific depending on the precipitation frequency (the period between rainfall events), intensity, duration, and size of the impervious area. The affects on urban hydrology include increased volume, frequency, and duration of runoff, larger peak discharges, larger flow velocities, changes in base flow regimes, and increased risk of flooding (Jones et al., 2005). Therefore, removing stormwater quickly and efficiently is a priority. Urban stormwater typically enters purpose-built infrastructure that discharges it to either a local waterway or a designated stormwater system or combined stormwater/wastewater system for treatment and discharge elsewhere. However, stormwater can transport urban contaminants at concentrations frequently above regulatory limits (Table 2-1) resulting in the degradation of local waterways, when discharged into these environments. The most common contaminants found in stormwater runoff include sediments, organic micropollutants (most commonly, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)), pathogenic microorganisms, nutrients, and heavy metals (Barbosa et al., 2012). These contaminants build up on urban surfaces because of anthropogenic activity and wash off in stormwater during precipitation.

Table 2-1. Typical concentrations of common heavy metals in stormwater and guideline concentrations for the 90% protection of aquatic species in Australia & New Zealand (ANZECC, 2000; Göbel et al., 2007).

Contaminant	**Mean heavy metal concentrations in stormwater** µg/L	**Australian and New Zealand Environmental and Conservation Guidelines for 90% protection level of freshwater aquatic species** µg/L
Zinc	1409.8	15
Copper	62.0	1.8
Lead	216.5	5.6

2.1.1 Contaminant Sources in Stormwater

Stormwater transports a wide range of organic and inorganic contaminants. Table 2-2 arranges the most common contaminants into six specific groups; however, this is a simplified view, as there can be a vast range of other contaminants that can be environmentally detrimental. Heavy metals are inorganic compounds with an atomic weight between 63.5 and 200.6 g/mol and a specific gravity greater than 5.0 that can occur in stormwater in either a dissolved (<45 µm) or particulate (>45 µm) form (Fu et al., 2011; Ahmed et al., 2016). Heavy metals, regardless of form, are a significant concern in stormwater because of their bioavailability and toxicity to aquatic ecosystems; this is especially so because once in the environment heavy metals cannot be removed completely and tend to accumulate in living organisms, sediment, and soils. The most common heavy metals found ubiquitously in stormwater, with concentrations frequently above regulatory guidelines, are zinc, copper, and lead (Seelsaen et al., 2006).

There are two main sources of heavy metals in stormwater: the transport sector (including footpaths, parking lots, feeder streets, major roads, highways, and vehicles) (Huber et al., 2016) and building materials (including roofing, downpipes, guttering, and flashings) (Göbel et al., 2007).

Infrastructure associated with the transport sector can constitute a vast area of imperviousness, allowing it to collect large concentrations of contaminants from vehicles and surrounding land uses. These factors make it the major source of heavy metal contaminants in a catchment (Davis et al., 2001a; Wicke et al., 2012). Transport infrastructure accumulates contaminants directly from vehicle use but also from the atmospheric deposition of contaminants from nearby sources (for example, industrial activities and exposed soils) (Charters et al., 2016). The build-up of heavy metals closely correlates to the average daily traffic volume, the volume capacity ratio, surface texture and depth, and antecedent conditions (Wicke et al., 2012). Furthermore, contaminants emitted by vehicles can disperse into the atmosphere and can travel for some distance before depositing on other impervious surfaces. The heavy metals deposited on impervious surfaces and found in the atmosphere are predominantly in a particulate form (Zanders, 2005; Charters et al., 2016; Borris et al., 2016)

Table 2-2. Characterisation of stormwater contaminants (modified from Barbosa et al., 2012)

Contaminant group	Measurement parameter	Sources
Solids	TSS	Pavement wear, construction sites, or rehabilitation works, atmospheric deposition, and anthropogenic wastes.
Heavy metals	Most commonly Zn, Cu, and Pb	Vehicle components, tyre wear, fuel and lubricating oils, traffic signs, and metallic road structures.
Biodegradable organic matter	BOD and COD	Vegetation, animals (cats, dogs, and birds, either fecal matter or dead bodies).
Organic micropollutants	PAHs, PCBs, MTBEs, endocrine disrupting chemicals	Incomplete fossil fuel combustion, abrasion, and wear of asphalt and tyres.
Pathogenic microorganisms	Total coliforms	Wildlife and domestic animals.
Nutrients	Nitrogen, phosphorous	Fertilisers and atmospheric deposition.

Roofing materials, and more generally building materials, are the second major source of heavy metals in urban stormwater, contributing heavy metals at concentrations frequently above regulatory limits. Impervious surfaces naturally are comprised of a significant proportion of buildings; for example, in New Zealand, the residential roofed area can make up as much as 20 – 25% of the total impervious suburban surface area (Brown et al., 2006). Roofing materials themselves can be considered a source of heavy metals in two ways; however, the contribution of heavy metals and other contaminants from roof surfaces to stormwater runoff is dependent on material, age, slope, exposure, and location (Wallinder et al., 2000; Göbel et al., 2007). Firstly, all roofing materials, along with guttering and downpipes can form corrosive products. This is due to corrosion processes on their surface when exposed to the prevailing atmospheric conditions (e.g. wind, rain, temperature, and humidity) which also determine the nature and composition of the products (He et al., 2001; Göbel et al., 2007; Veleva et al., 2010). Once formed, corrosion products can be removed in two ways, physically by wind and erosion and secondly by dissolution, ultimately resulting in the contamination of stormwater. Secondly, contaminants from atmospheric deposition frequently settle on roofs adding to the concentration of contaminants; atmospherically deposited contaminants can come from a variety of sources, for example, the transport sector, industrial activity, fertilisers and pesticides.

2.1.1.1 Zinc

Zinc, in trace concentrations, is essential to all life on earth; however, in high concentrations it can be toxic to organisms causing a range of reproductive, developmental, and behavioural disorders (Councell et al., 2004). Anthropogenic sources of zinc include metal production, fossil fuel consumption, phosphate fertiliser, deicing salts, galvanised railings, fuel and oil, brake linings, and rubber tyres (Davis et al., 2001a; Councell et al., 2004). Specifically, zinc is used as a vulcanising agent in the manufacturing of tyre rubber. It can also be found in brake linings, fuel, and oil. Tyres can contain concentrations of zinc in the range of 1.19 – 18.30 g/kg, which can equate to approximately 25% of the total zinc input for a catchment (Davis et al., 2001a; Zanders, 2005). Dust produced from brake wear can contain concentrations of zinc between 0.35 – 9.63 g/kg but contributes overall less (approximately 3%) zinc to a catchment. Zinc-galvanised building materials (roofing, guttering, downpipes, and nails) are some of the most common materials used nowadays, utilised primarily in commercial and industrial buildings because of their low-maintenance and long lifespans. Nevertheless, their use in residential buildings is also common. Steel and iron roofing materials will corrode and rust if not coated (galvanised) with zinc prior to use. Zinc is a very active metal (standard redox potential of -0.76V) which corrodes easily when exposed to the atmosphere; this corrosion creates an oxide/hydroxide barrier layer between the metal and the atmosphere ultimately protecting the galvanised material (Veleva et al., 2010). The solubility properties of the corrosion products ultimately determine their dissolution during precipitation; for example, $ZnSO_4$ is a highly soluble zinc salt that dissolves readily during periods of precipitation (Veleva et al., 2010). Runoff concentrations of zinc are dependent on the roofing material but can range anywhere between 0.1 – 10.0 mg zinc/L (Davis et al., 2001a; Heijerick et al., 2002) and do not differ significantly with time (Veleva et al., 2010).

2.1.1.2 Copper

A major source of copper in the transport sector is the degradation of brake pads and given their relative importance, abundance, and continual wear, they can contribute substantial amounts of copper to stormwater runoff. In fact, brake pads can contribute up to 47% of a catchment's copper component (Davis et al., 2001a). Copper roofs and building materials are without a doubt the biggest contributors of copper contamination in stormwater. While larger buildings, for example, churches and halls, traditionally use copper roofing materials, these days more and more domestic homes use copper as a more visually appealing alternative to galvanised steel. Leaching rates of copper from these materials can depend on rainfall quality and quantity and the proportion of copper material used. Pennington et al. (2008) recorded copper concentrations from full copper roofs of up to 7690 µg/L, the majority of which was found in dissolved form; whereas, copper concentrations from copper guttering were found to be approximately 590 µg/L.

2.1.1.3 Lead

The origins of lead, largely found in the transport sector, are associated with leaded gasoline and wheel balancing weights. Concentrations of lead in the environment peaked in the 1970s when the use of leaded gasoline was at its maximum; however, lead concentrations have since declined by as much as 10 to 100 times, since its phasing out (Davis et al., 2011). Given leads affinity to particulates, roadside soil and dust can be a significant source of lead as the result of contamination over previous decades (Mosley et al., 2001).

2.1.2 Heavy Metal Partitioning

The partitioning of heavy metals between dissolved (<0.4 µm) and particulate (>0.4 µm) form can have important implications for the ecotoxicity of stormwater entering waterways and ultimately the efficiency of any treatment applied (Mosley et al., 2001). Dissolved heavy metals are considered bioavailable and can be readily assimilated by aquatic biology causing acute toxicity. They are also the most difficult to treat because of their dissolved nature. The partitioning of heavy metals is dependent on a range of environmental conditions. However, the reactions occurring at the surface of particles, for example within stormwater treatment systems, can play a dominant role in heavy metal partitioning by converting dissolved heavy metals to particulate-bound heavy metals.

Removal of a dissolved metal ion from solution requires either that the ion be 'precipitated' from the solution or adsorbed to the surface of a solid. Solids carry a charge when in an aqueous environment, and as a result, they attract dissolved ions of the opposite charge to balance that charge. Balancing reactions can also be referred to as sorption reactions; these reactions attract dissolved ions and bind them to a surface, ultimately converting them to particulate bound ions. The binding process can be highly competitive between ions, as binding sites on particles can be limited. In this way, particulate bound metals can act as a significant reservoir for heavy metals if the prevailing environmental conditions change resulting in concentrations of dissolved heavy metals constituting a significant proportion of total contaminant load (Gnecco et al., 2008). Precipitation, on the other hand, occurs when chemical reactions in a solution cause the formation of solids when the system becomes supersaturated (Lewis, 2017). Precipitation is a highly pH dependent process and is considered irreversible.

The speciation of the dissolved forms of a heavy metal further influence the toxicity of stormwater, with free ion forms of a metal considered the most bioavailable and thus toxic to aquatic environments (Mosley et al., 2001; Warren et al., 2001; Dean et al., 2005). For example, a stormwater with high concentrations of heavy metal may be less toxic than one with lower concentrations of heavy metals if the partitioning of heavy metals within the latter stormwater makes them more bioavailable when compared to the former stormwater. A study by Dean et al. (2005) found that within the dissolved fraction, ionic forms of zinc, copper, lead made up 53%, 38%, and 5% respectively of the total dissolved

concentration, meaning that high concentrations of metals are available to aquatic organisms if discharged into waterways. These differences in the dissolved concentration of zinc, copper, and lead occur due to differences in removal mechanisms, for example, zinc is mainly retained by adsorption whereas copper and lead are retained by a combination of adsorption and precipitation (Gülbaz et al., 2015). Thus, in general, as much as 94% of the total concentration of zinc in stormwater is found in the dissolved form; whereas, copper can be found in both particulate and dissolved form, the latter of which may comprise 40 – 80% of the total concentration in stormwater (Pennington et al., 2008). Lead on the other hand is primarily particulate-bound, of which it makes up 95 – 99% of the total load, with very little found in dissolved form (Borris et al., 2016).

Thus, zinc, copper, and lead show varying affinities for solids, which ultimately determines the concentration of their dissolved form in stormwater and their bioavailability within waterways receiving stormwater. It is therefore crucial that stormwater treatment systems target the most ecotoxic form of heavy metals to effectively reduce and prevent environmental degradation.

2.2 Stormwater Management

2.2.1 Traditional Stormwater Management

The aim of stormwater management is to protect human and ecological values by preventing and mitigating the adverse effects of stormwater on the human and natural environments (ARC, 2003). Urbanisation is commonly associated with increases in impervious surfaces resulting from building and transport infrastructure. Large impervious surfaces are responsible for large surface water flows (higher peak flows and changes in base flow) as well as longer and more frequent flooding during and after rainfall events. Traditional stormwater management focused on removing peak flows away from people, buildings, and transportation systems as quickly and efficiently as possible to maintain infrastructure functionality and public health (Jones et al., 2005; Barbosa et al., 2012). In order to accomplish this, structural conveyance networks (e.g. kerb and channel, pipe networks, and paved areas) that concentrated runoff flows for discharge were established. These systems typically discharge their contents into combined sewer systems, which transport wastes to treatment facilities or directly into the nearest waterway (Jones et al., 2005; Barbosa et al., 2012). With increasing urbanisation and larger return period storms, traditional combined sewer systems became subject to frequent overflows, in which untreated wastewater and/or stormwater is discharged into local waterways. Thus, this system of stormwater management has increasingly become unsustainable as the ecological health and integrity of waterways is compromised (Roy et al., 2008; Barbosa et al., 2012). Furthermore, the construction of stormwater infrastructure was predominantly based on economic factors not environmental factors and the need to remove water quickly and as efficiently as possible (Barbosa et al., 2012). As a result, urban

areas can lack integrated transportation and land-use planning resulting in inefficient stormwater management systems and increased environmental impacts (Jones et al., 2005).

2.2.2 Stormwater Management in New Zealand

New Zealand manages its natural and physical resources through a variety of legislation. The Resource Management Act 1991 (RMA) is New Zealand's foremost piece of planning legislation, and the first in the world, that attempts to implement principles of sustainable development. The RMA embraces environmental impact assessments and requires that adverse environmental affects of an activity be avoided, remedied, and mitigated at all stages of development (Zanders, 2005; Berke et al., 2006). Also particularly relevant, especially to stormwater management, are the guidelines set by the Australian and New Zealand Environment and Conservation Council (ANZECC). This council has developed a range of national water quality management strategies with guidelines that provide a framework for identifying and protecting water quality for a range of environmental values (ANZECC, 2000). While the guidelines are not a legal statute, they offer guidance to national and local authorities on a wide range of chemical, physical, and biological parameters and/or indicators and provide threshold values to protect water quality in a range of aquatic environments (ANZECC, 2000). The ANZECC guidelines provide 'trigger' values, which indicate a potential environmental problem that warrants a management response (e.g. further investigation); however, it is important to be aware that aquatic environments can vary significantly from one ecosystem to the next. Thus, the trigger values are set at four different protection levels, 80, 90, 95, and 99% protection (protection levels indicate the percentage of species expected to be protected at that trigger value); with the decision to apply a certain protection level the prerogative of local authorities (ANZECC, 2000). Stormwater management requires consideration of both the RMA and ANZECC guidelines, and as a result, district councils are required to adopt and implement plans that promote the sustainable management of their natural and physical resources (Berke et al., 2006). The 1980s and 1990s saw a move towards more sustainable stormwater management, where councils developed management strategies which maintained stormwater removal for public health and emphasised environmental health, from an economic and environmental perspective. Their aim was to replace uneconomic and non-environmentally friendly piping, channels, and other structures, which have high rates of depreciation and high replacement costs, with natural treatments.

2.3 Low Impact Developments

The recognition of stormwater runoff as an agent for environmental damage led to the need for new stormwater management objectives. However, while source control is important it can be difficult due to the large areas of imperviousness and the vast range of contaminants and their sources. Therefore, Low Impact Developments (LIDs) were developed and piloted in Prince George's County, Maryland in the 1990s to treat stormwater before discharge (Ahiablame et al., 2012; Liu et al., 2014). LIDs are a land planning and engineering design approach that encompass a range of structural practices, which promote onsite detention and infiltration to compensate for land development impacts. Practices include bioretention systems, infiltration trenches, wetlands, swales, green roofs, vegetated buffer strips, and cluster designs (Dietz, 2007; Ahiablame et al., 2012). In addition to LIDs, various stormwater treatment systems have been established throughout the world according to the focus and the country in which they were developed; for example, Sustainable Urban Drainage Systems, are common in the United Kingdom and Water Sensitive Urban Design in Australia (Barbosa et al., 2012). Stormwater treatment systems have been developed as a way of maintaining an area's post-development hydrology to near natural conditions, ultimately reducing the need for paving, kerb and channel, pipe systems and inlet structures, all of which can have negative affects on the area's natural hydrology and visual aesthetics (Ahiablame et al., 2016). Furthermore, the installation of stormwater treatment systems reduces peak flows, runoff volumes, and stormwater pollution, while promoting groundwater recharge, and the maintenance of stream base flows (Charlesworth et al., 2003). When compared to old stormwater management practices, modern stormwater treatment systems promote filtration and infiltration processes, onsite storage and detention, evapotranspiration, sorption, precipitation, biodegradation, phytoremediation, and percolation as means of treating and controlling stormwater (Ahiablame et al., 2012). The following are a range of stormwater treatment systems common in New Zealand (modified from Ahiablame et al., 2012).

- Bioretention systems – (also known as rain gardens or biofilters) are shallow depressions in the landscape that treat and attenuate stormwater.
- Swales/infiltration strips – a shallow open channel of either grass or vegetation that conveys water and allows it to infiltrate the ground.
- Detention basins – a dry depression of land designed to treat a specific volume of water.
- Green roofs – a building's roof completely or partially covered with vegetation over a drainage layer.

All types of stormwater treatment systems employ a range of contaminant removal mechanisms that work to improve water quality; however, for ease, only contaminant removal in bioretention systems will be discussed.

2.3.1 Contaminant Removal in Bioretention Systems

Bioretention systems are shallow depressions in the landscape that treat and attenuate stormwater. Their basic design consists of layers of mulch, filter material, and gravel, of varying depths depending on target treatment (Figure 2-1). The filter layer is the largest layer that can be between 0.7 – 1 m deep, and it typically incorporates a soil-based material (e.g. sandy loam) with organic matter and any other materials that may increase the sorption properties of the system (e.g. limestone or perlite) (Davis et al., 2009; Lim et al., 2015). The composition of the filter layer is crucial to the overall performance of the system. As stormwater percolates through the media, its composition can influence the system's ability to drain water (hydraulic conductivity), remove contaminants, and grow vegetation (Ahiablame et al., 2012; Lim et al., 2015). A gravel layer at the lowest level of the system can surround an underdrain. An underdrain may be a preferable option if the surrounding soils have low permeability. While not all bioretention systems have an underdrain, if present, the underdrain discharges stormwater into a designated stormwater or wastewater system or a local water body (Li et al., 2008; Lim et al., 2015). Finally, a hardwood mulch layer on the very top keeps the system moist (Lim et al., 2015).

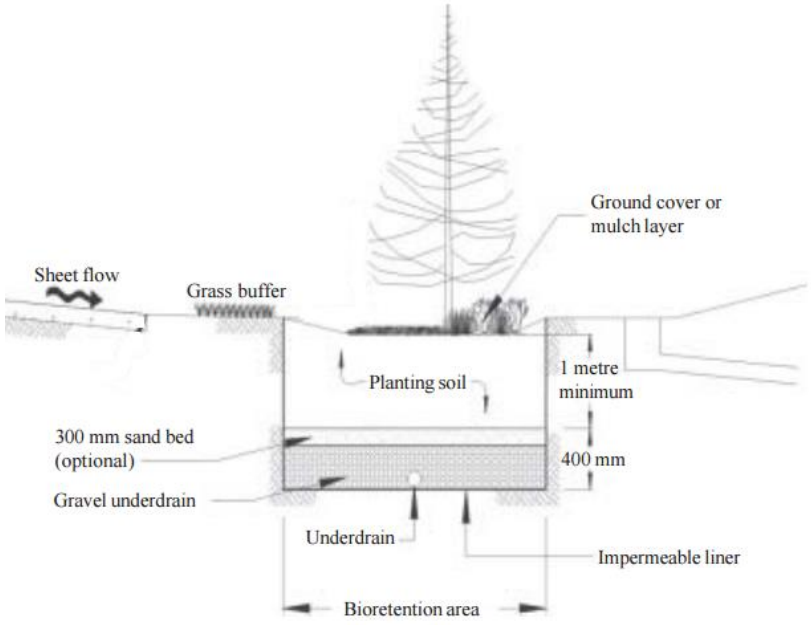

Figure 2-1. Typical bioretention design (ARC, 2003).

Grasses, shrubs, and small trees promote evapotranspiration, contaminant removal by sorption and biological activity while helping to maintain soil properties and hydraulic conductivity (Li et al., 2008). As the runoff infiltrates the bioretention system a range of mechanisms remove urban contaminants, including physical (sedimentation, filtration, sorption, ion exchange, phytoremediation, precipitation, and volatilization) and biological (microbial activity, plant uptake, decomposition, and thermal attenuation) processes (Li et al., 2008; Fassman et al., 2013).

Studies on the performance of bioretention systems have demonstrated their ability to retain and transform heavy metal and nutrient contaminants in both laboratory and field settings (Table 2-3). Heavy metal removal in bioretention systems can vary between 30% and 99%, with the majority of heavy metals captured in the top surface layers. Depending on the partitioning of heavy metals, sedimentation and filtration will effectively remove particulate bound metals while sorption, ion exchange, precipitation, and microbial or plant uptake will remove dissolved fractions. The treatment of dissolved heavy metals by bioretention systems is dependent on a range of environmental conditions, for example, environmental pH, temperature, the filter media used in the construction of the bioretention system, and the presence or absence of vegetation and microbial activity. pH can significantly alter the concentration of particulate or dissolved metals in stormwater, which can have major implications for stormwater treatment by bioretention because it relies on contaminant removal primarily through sedimentation and filtration (Good et al., 2014). The pH of incoming runoff depends on the pH of the atmosphere and the surfaces over which it has flowed; however, urban runoff typically has a pH in the range of 5 – 7 (Pennington et al., 2008; Good et al., 2014).

A pH of between 5 and 7 is sufficient to promote metal dissolution in runoff and enhance metal mobility thereby reducing the efficiency of bioretention systems to remove urban contaminants. A field study by Li et al. (2008) found that within the soils of bioretention systems, zinc can remain highly mobile compared to copper and lead, and that metal mobility increased as the pH of the substrate decreased. Therefore, if the pH of the bioretention system is low enough it can support dissolved heavy metals, further reducing the overall treatment efficiency of the system. Furthermore, experimental results from Davis et al. (2001b) verified that both soil and mulches have an affinity for heavy metals; at a neutral pH metal sorption by filter material was greater than 60%, 80%, and 90% for zinc, copper, and lead respectively; however, at a pH of 4 little sorption was observed.

Bioretention systems also commonly contain plant vegetation, which in addition to sedimentation and filtration can provide heavy metal treatment opportunities. For example, plant vegetation affects metal solubility and mobilisation by influencing dissolved organic matter content and pH (Blecken et al., 2011). Furthermore, plants have the ability to directly take-up metals from their environment, accounting for as much as 10% of the total metal removal (Davis et al., 2001b; Blecken et al., 2011). Vegetation can also be beneficial in promoting microbial activity in that it provides an ideal environment within the soil for microbial survival.

Table 2-3. Summary of percent contaminant removal by bioretention systems.

Nature of Study	Location	TSS	NO_3-N	NH_4-N	TN	TP	Zn	Cu	Pb	Reference
Field	Maryland, USA	47	83	-	-	76	62	57	83	Davis (2007)
Laboratory	Sweden	98	-944	74	-172	91	-	-	-	Blecken et al. (2010)
Laboratory	Sweden	98	-	-	-	-	98	93	96	Blecken et al. (2011)
Laboratory	Maryland, USA	-	24	60 - 80	-	60 - 80	>92	>92	>92	Davis et al. (2001b)
Field	Connecticut, USA	-	35	84.6	32.0	-110	-	-	-	Dietz et al. (2005)
Laboratory	Maryland, USA	-	-	-	-	-	88 - 97	88 - 97	88 - 97	Sun et al. (2007)
Field	Maryland, USA	-	>15	-	>49	>65	43 - 97	70 - 95	64 - 95	Davis et al. (2003); Davis et al. (2006)
Laboratory	Maryland, USA	-	1 - 43	2 - 49	-	4 - 99	-	-	66 - >98	Hsieh et al. (2005)
Field	North Carolina, USA	-	13 - 75	-	-	-	98	99	81	Hunt et al. (2006)
Field	North Carolina, USA	60	-	73	32	31	77	54	31	Hunt et al. (2008)
Laboratory	Christchurch, New Zealand	-	-	-	-	-	61 - 81	0.3 - 56	81 - 90	Good et al. (2012)

2.3.2 Alternative Media for Contaminant Removal

Recently, the use of readily abundant and recycled materials has been investigated for use in stormwater treatment. This would enable designers to utilise alternative materials at a relatively low cost and the recycling of a material would add value to its lifecycle. Materials of most interest demonstrate a capacity to remove/treat contaminants and include, compost, iron oxide-coated sand, recycled glass, zeolites, limestone, mussel shell, rice bran and hulls, soybean, peanut husk, sawdust, and peat (e.g. Bailey et al., 1999; Seelsaen et al., 2006; Good et al., 2014; Grace et al., 2016)

Mussel shells are an abundant, alkaline, waste product from the shellfish industry that have been shown to be useful for the removal of heavy metals from stormwater (e.g. Good et al., 2014). Their primary mechanism of removal is pH buffering. When mussel shells are added to bioretention systems they increase the pH of the system preventing the dissolution of particulate-bound heavy metals, allowing the particulates, and associated heavy metals, to be filtered out through the bioretention media. Thus, mussel shells increase the pH of bioretention systems ultimately allowing these systems to treat stormwater more efficiently through sedimentation and filtration. A laboratory study by Good et al. (2014) found an increase in the removal efficiency of zinc from 55%, in systems with no mussel shell, to 80% in systems that contained mussel shell, demonstrating an increased metal removal efficiency with increasing mussel shell volume. Similar results were found for copper (27% removal with no mussel shell to 47% removal with mussel shell), however, lead was removed efficiently from all systems given its prevalence in particulate form (Good et al., 2014).

Recycled glass is a municipal waste product that is comprised predominantly of packaging containers for food and drink, as well as, glass from household items (e.g. glassware and ornaments), and sheet glass or glass from demolition activities (Arulrajah et al., 2015). Glass is, generally, 100% recyclable, as it can be crushed, heated, and remoulded into new products. Despite this, limitations exist in the processing abilities of the glass recycling industry (Arulrajah et al., 2015). Many studies have demonstrated the potential for recycled glass to be utilized in wastewater treatment as an alternative to sand, for example, in mono- and dual-media filters and recirculating biofilters (Hu et al., 2006; Horan et al., 2007; Gill et al., 2009; Petrella et al., 2009; Soyer et al., 2010). Recycled glass has also been shown to be as effective as sand in slow and rapid filtration systems and granular filtration for potable water treatment (Rutledge et al., 2002; Soyer et al., 2010; Davies et al., 2012; Soyer, 2016). Published studies on the use of recycled glass in stormwater treatment are limited to the work of Seelsaen et al. (2006). Seelsaen et al. (2006) examined the contaminant removal abilities of a range of alternative filtration media for stormwater treatment, including recycled glass. Differentiating between fine and coarse glass, they found that fine glass had zinc and copper removal capabilities of 70% and 40% respectively, while coarse glass had zinc and copper removal capabilities of just 16% and 26% respectively. Crushed recycled glass, while inert, has demonstrated an ability to sorb common

contaminants from stormwater offering an alternative and innovative use of recycled glass in stormwater treatment.

Both mussel shell and recycled glass have shown promising heavy metal treatment capabilities; however, there is a lack of supporting literature and thus there is a need to better understand their role in dissolved heavy metal removal from stormwater.

2.4 Biofilm

A biofilm is a collection of microorganisms attached to a moist abiotic surface and enclosed in a matrix of extracellular polymeric substances (EPS) (Donlan, 2002; Singh et al., 2006). Biofilm formation (Figure 2-2) occurs when free-living microorganisms attach themselves, through physicochemical interactions or protein secretions, to a surface. The interface between a solid surface and an aqueous medium (e.g. water) provides an ideal environment for microorganism attachment and growth and within minutes, the microorganisms proliferate, and other microorganisms then join to form a microcolony. A variety of environmental factors regulate the formation of biofilm; including the texture and/or roughness of the substratum, flow velocity, pH, and temperature (Hu et al., 2005).

Biofilms offer a mechanical strength to microorganisms, which is not available to their free-living counterparts; this protects the biofilm community from shear forces, nutrient deprivation, and many environmental changes (e.g. pH changes) (Singh et al., 2006). Furthermore, the composition of microorganisms within a biofilm is constantly changing in time and space in response to internal and external processes, and as a result, biofilms can contain a range of different species, which can make up microcolonies within the biofilm itself (Donlan, 2002). The secretion of EPS encloses the microorganisms and forms a mature biofilm. The EPS can constitute 50% to 90% of the total organic carbon of the biofilm and provides protection during periods of stress (Donlan, 2002; Feder et al., 2015). The microorganisms present, the nutrients available, and the prevailing physical conditions determine the composition of EPS; despite this, the EPS is predominantly composed of water, up to 97%, followed by microbial cells, polysaccharides, and protein (Sutherland, 2001). In many cases, it can also contain particulate material and debris from the surrounding environment.

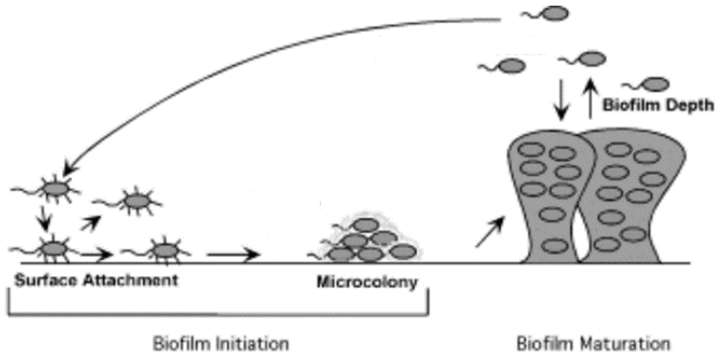

Figure 2-2. A diagram of biofilm formation on a solid surface (modified from Stanley et al., 2004)

Soil can provide a stable substrate for the growth of biofilms, which in turn play an important role in the physicochemical makeup of the soil. Within soils, biofilms form on and between soil particles, attaching themselves to particles using EPS, to create a 'bioweb' type structure. This bioweb structure is comprised of different sized clusters of biofilm, depending on the liquid flow velocity, that are connected by networks of fibrillary strands (Rodríguez et al., 2007). Soil systems provide a large surface area and plenty of pore space through which biofilms can form; the soil also provides nutrients and offers protection during changing environmental conditions (Rodríguez et al., 2007). Despite intermittent wetting, moisture is maintained by surface tension (around gravel particles and/or between pore spaces) between rainfall events, the substrate is stable and nutrient concentrations are high making stormwater treatment systems an ideal environment for the growth of biofilm (Feder et al., 2015).

2.4.1 Biofilms and Heavy Metal Removal

Biofilms possess a range of properties that make them highly effective at removing heavy metals from metal-contaminated water (Feder et al., 2015). Together the EPS and the microorganisms within the biofilm can facilitate the sorption of heavy metals through passive (biosorption) and active (bioaccumulation) cellular processes. These processes are possible because of the functional groups found in both the EPS and in the cell walls of microorganisms (e.g. amino, carboxylic, hydroxyl, phospholipids, proteins, and polysaccharides) (Hu et al., 2005; Feder et al., 2015). Biosorption is a quick process that occurs independently of the cell's metabolism making it a passive process. Biosorption is a simple physicochemical phenomenon in which the binding of metal ions to the functional groups of a cell's wall can occur by any one or a combination of the following processes: physical adsorption, ion exchange, van der Waals forces, complexation, and precipitation (Hu et al., 2005; Hansda et al., 2016). Bioaccumulation is an active process because it is metabolism dependent. Bioaccumulation occurs in two stages, the first is identical to biosorption, and the second involves the transport of bound metal ions into the cell membrane (Hansda et al., 2016).

While soil, mulch, and vegetation in bioretention systems contribute to the removal of particulate heavy metals from stormwater by sedimentation and filtration, dissolved heavy metals can remain untreated. Thus, high concentrations of dissolved heavy metals are common in stormwater outflows from these systems. Biofilms have a natural affinity for dissolved metals and thus could offer a means to improve the bioremedial properties of bioretention systems by reducing concentrations of dissolved heavy metals in stormwater outflows. Few studies have investigated the ability of biofilms to assimilate heavy metals in bioretention systems. Feder et al. (2015) physically and numerically modelled the influence of biofilms on heavy metal removal in gravel filters in laboratory experiments. Breakthrough column experiments showed that gravel filters could remove up to 51% of metals passing through them; however, columns with biofilm growth significantly enhanced metal removal by a further 29%. Furthermore, the composition of biofilm differed depending on the lithology of the substrate but did not show any significant difference in their ability to assimilate heavy metals (Feder et al., 2015), illustrating that bioremediation will occur regardless of substrate or biofilm composition. Ancion et al. (2010) on the other hand used flow chamber microcosms to show that metals can accumulate quickly and to high concentrations in biofilm, with the most significant accumulation occurring during the first 7 days, after which accumulation plateaued (Figure 2-3). Additionally, they showed a positive relationship between metal concentrations in water and concentrations of the same metal in the biofilm (Figure 2-4). Enrichment factors of up to 500:1, 1500:1 and 6000:1 for zinc, copper, and lead respectively were recorded after exposure to moderately contaminated synthetic stormwater (500, 50, and 50 µg/L of zinc, copper, and lead respectively) for 21 days (Ancion et al., 2010).

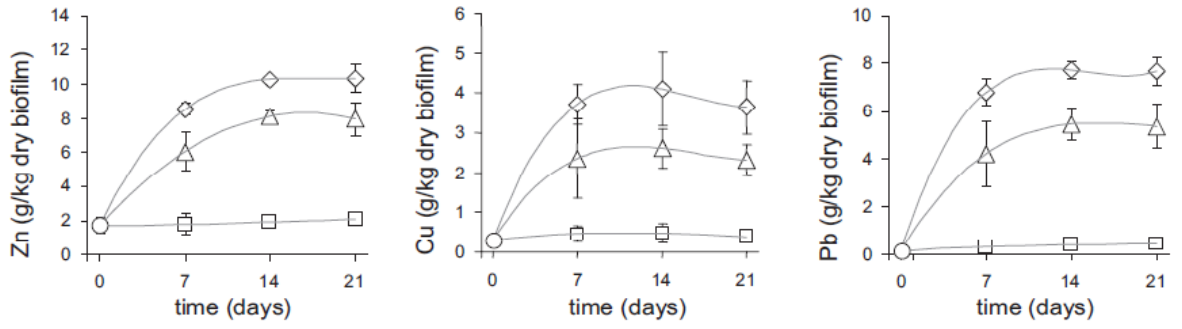

Figure 2-3. Concentrations of zinc (Zn), copper (Cu), and lead (Pb) in biofilm over 21 days. Data are (○) concentration at the beginning of each experiment, (□) concentrations after exposure to no additional metals, (Δ) concentrations after exposure to moderately contaminated synthetic urban runoff, and (◊) concentrations after exposure to highly contaminated synthetic urban runoff (Ancion et al., 2010).

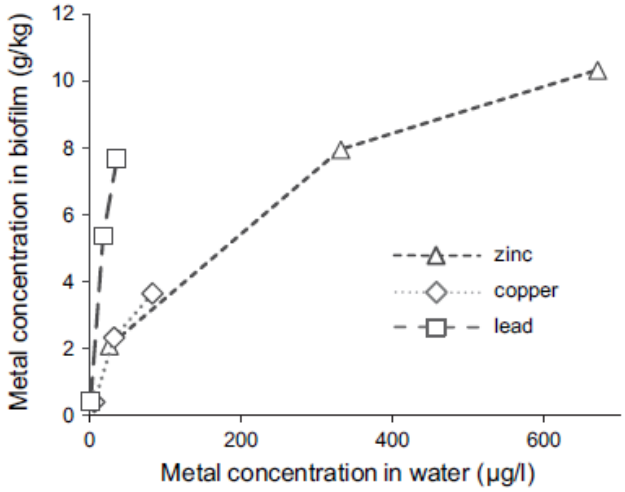

Figure 2-4. Concentrations of heavy metals in biofilm against the concentration of the same heavy metal dissolved in water after 21 days exposure (Ancion et al., 2010).

2.5 A Need for Research

Research has demonstrated the ability of biofilms to sorb heavy metals from aqueous environments and current understanding would suggest that the presence of biofilms in bioretention systems may improve the removal of heavy metals from stormwater. In particular, dissolved heavy metals, which have the potential to escape treatment, and be discharged into receiving waterways, ultimately resulting in environmental degradation. Thus, there is a need to understand the role of biofilms in the sorption of dissolved heavy metals from stormwater in an effort to improve the efficiency of bioretention systems by enhancing dissolved metal uptake to prevent environmental degradation.

Zinc, copper, and lead show varying affinities for solids, which ultimately determines the concentration of their dissolved form in stormwater and their bioavailability in waterways receiving stormwater. Therefore, it is crucial that stormwater treatment target the dissolved and most ecotoxic form of heavy metals to effectively reduce and prevent environmental degradation.

Chapter 3. Methods

To ascertain the effectiveness of biofilms and substrate selection in absorbing heavy metals from stormwater, experiments were organised as described next. To begin, the stock biofilm that would be used in the first phase of experiments was grown (section 3.1), after which two phases of experiments were run. The first phase of experiments (phase one, section 3.3.3), layered biofilm experiments, were designed to investigate the dissolved heavy metal removal efficiency of two different types of biofilm. The second phase of experiments (phase two, section 3.3.4), whole column biofilm experiments, were designed from the results of the layered biofilm experiment, whereby dense autotrophic biofilm was grown in the columns before they were dosed with synthetic stormwater. Gravel experiments were then run as a control (section 3.4.1) and batch adsorption experiments were conducted for all three substrates to confirm the capacity of the three substrates to adsorb dissolved heavy metals (section 3.4.2). All laboratory experiments were conducted at the University of Canterbury's Environmental Engineering Laboratory in the Department of Civil and Natural Resources Engineering.

3.1 Biofilm Stock Growth

Biofilm stock was grown in plastic Polymethylpentene (PMP) 1000 mL measuring cylinders (columns). Two columns were prepared with an inflow valve at the bottom and an outflow at the 800 mL level (Figure 3-1 and Figure 3-2 A and B). The columns were filled with approximately 700 mL of recycled glass. Recycled glass was chosen as the growth substrate because it is an inert substrate that would not influence the growth of biofilm, while offering an optimal surface area for the biofilm to grow on. One column was entirely wrapped in tinfoil to prevent light infiltration for the duration of the experiment, while the other column was exposed to light. By doing this, two different compositions of stock biofilm were expected to grow; predominantly heterotrophic bacteria were expected to grow in the column without light infiltration and autotrophic bacteria and algae were expected to predominate in the uncovered column. To better replicate their natural environments (e.g. rivers, streams, and pipes), the growth solution (section 3.1.1) was cycled through the columns as follows. One end of the inflow tube was placed into the solution reservoir and the other end connected to the inflow valve in the bottom of the column. The growth solution was pumped from the solution reservoir using a peristaltic pump. The outflow tube, at the 800 mL level, drained the system, returning the solution to its original solution reservoir. Thus, the solution was circulated through the column from the bottom upwards, for a period of approximately 4 months, after which dense biofilm growth was observed.

Prior to using the biofilm stock in the experiments, the biofilm and its recycled glass substrate were transferred to flat containers (Figure 3-2 C). With the addition of inflow and outflow valves at either end of each flat container, the growth solution continued to circulate through the system. Transferring

them to flat containers allowed for better access and enabled the biofilm stock to be moved to the experimental columns with minimal disturbance. After moving the biofilm to flat containers, they were additionally dosed with nutrients (Table 3-1) and left to re-establish for 2 weeks before the start of the experiments.

3.1.1 Growth Solution

Water collected from Haytons and Okeover Streams was circulated through the columns to promote biofilm growth. Scrapings of natural biofilm from Haytons Stream were collected and used to seed the columns to initiate the growth of biofilm. Each column had its own solution reservoir with equal parts of stream water and natural seed biofilm. Given the finite time frame of this research, dosing the stream-water solution with nutrients, carbon, and activated sludge was necessary to facilitate the growth of biofilm. Nutrient and carbon concentrations were added at concentrations approximate to those found in natural stormwater (Table 3-1). Activated sludge was collected from the Bromley Wastewater Treatment Plant in Christchurch, New Zealand. Activated sludge was added both to the solution reservoir and directly to the columns, to increase the microbial activity within the columns to further initiate the growth of biofilm.

Table 3-1. Nutrient and carbon concentrations added to biofilm stock solution and their sources (modified from Seelsaen et al., 2006; Blecken et al., 2010)

Constituent	Mean concentration (mg/L)	Source
Dissolved NO_x-N	0.4	Potassium nitrate (KNO_3)
Dissolved NH_4-N	0.22	Ammonium chloride (NH_4Cl)
Organic N	5	Nicotinic acid ($C_6H_5O_2N$)
Total Phosphorus	0.31	Potassium dihydrogen phosphate (KH_2PO_4)
Dissolved Organic Carbon	10	Sodium acetate (CH_3COONa)

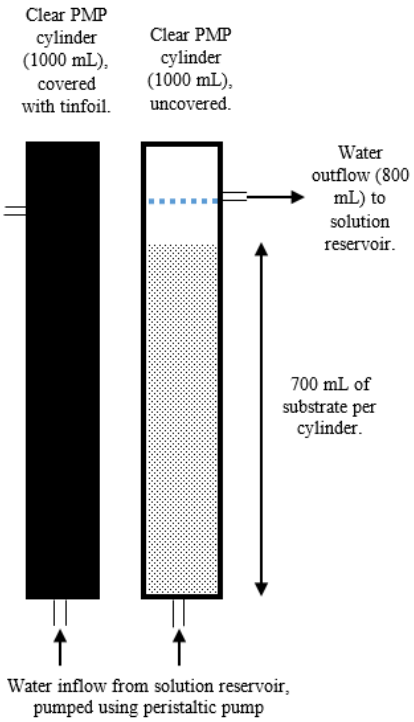

Figure 3-1. A diagrammatic representation of the laboratory setup used to grow stock biofilm. One column was covered with tinfoil to prevent light exposure, while the other column remained uncovered.

Figure 3-2. Laboratory setup for the growth of biofilm stock. (A) and (B) Initial biofilm growth occurred in columns; (C) biofilm and substrate were moved to flat containers to allow better access for experiments. Each column/container had its own solution reservoir.

3.1.2 Microscope Images

Images of the biofilm were taken in the Department of Biological Sciences at the University of Canterbury. Using the Nikon Eclipse 80i compound microscope, images (camera model was Nikon Digital Sight DS 5Mc) were taken of the biofilm at 20 and 40 times magnification. With expert help and reference books, identification of common and abundant microorganisms was possible; the abbreviation cf. is used to denote a probable identification.

3.2 Substrate Characterisation

3.2.1 Recycled Glass

Approximately 20 kgs of ≤ 16 mm crushed recycled glass (Figure 3-3 A) was sourced from manufacturers 5R Solutions in Hornby, Christchurch. Before use, the material was sieved (2.36 µm sieve) and washed with deionized water (DI water) to remove small particles and fine dust that may have obstructed the systems during experiments.

3.2.2 Mussel Shell

Mussel shell was sourced from Pearsons Landscape Supplies and came unwashed and roughly crushed (Figure 3-3 B). Before use the shell was further crushed and sieved, such that material passing through the 2.36 mm sieve but retained on the 1.18 mm sieve was put aside for use in the experiments (Figure 3-3 C). This process was repeated until sufficient material was obtained. The mussel shell was washed and dried before use in the experiments.

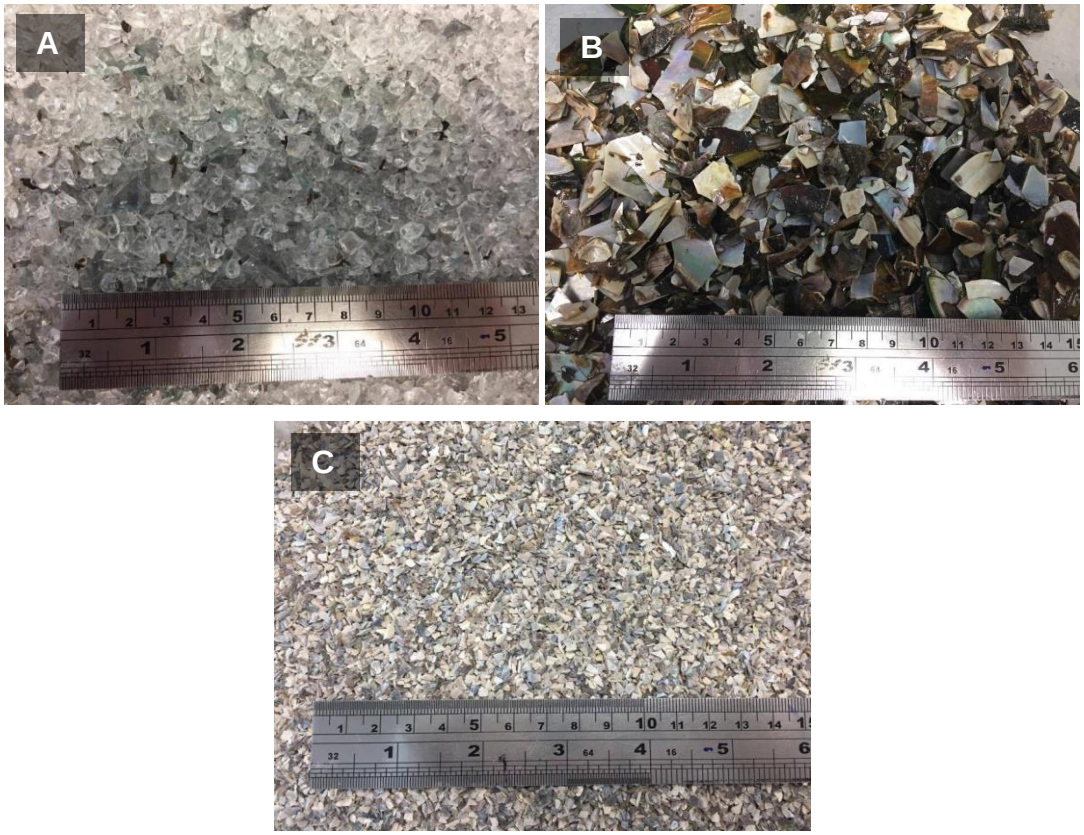

Figure 3-3. Substrates used in experiments. (A) recycled glass, (B) mussel shell, and (C) crushed mussel shell (size fraction > 1.18 mm – < 2.36 mm).

3.2.3 Substrate Analyses

3.2.3.1 Baseline contaminant concentrations

Prior to the commencement of the laboratory experiments, a single composite sample of each substrate was taken and analysed to determine background concentrations of contaminants. Samples of each material were added to individual beakers with DI water and left to soak, while being stirred, for a period of time after which a sample of the solution was taken and analysed by Inductively Coupled Plasma Mass Spectrometry (ICP MS) for dissolved zinc, copper, and lead.

3.2.3.2 Substrate pH

Given the influence of pH on metal speciation, benchtop experiments were run to determine the pH of each substrate. Experiments were run with equal volumes of the substrate (recycled glass or mussel shell) and DI water to create a 10% substrate solution (n = 3). For the duration of the experiment, the solution was mechanically stirred. Discrete samples of solution were taken at 1, 2, 4, 6, 10, 20, and 30 minutes to test for any changes in pH after which an average pH was calculated. Substrate pH was measured using calibrated EDT pH meters (for information regarding pH see section 3.5.2.1 and section 3.5.3).

3.2.3.3 Hydraulic conductivity

Both substrates were tested for hydraulic conductivity using a modified version of the constant-head saturated hydraulic conductivity method. Plastic sample containers were used (cross-sectional area of 1407.96 mm^2) with six holes drilled evenly in the bottom to allow drainage. Composite samples of the substrates were added to the containers at two different heights, 30 mm, and 60 mm. Samples were flushed approximately five times to remove any dust or small particles. After flushing, the experimental procedure described next was followed. Containers were held under a tap using a clamp stand, and then the tap, with a short hose attached to control flow, was turned on and adjusted to maintain a constant head of 20 mm over the substrate. When a constant head was achieved, the container was removed and the flow rate of water from the tap was determined using a stopwatch and a volumetric flask (n = 20 measurements per substrate). A deviation of Darcy's equation (equation 3-1) was applied to calculate the constant-head saturated hydraulic conductivity for each substrate.

$$K_{sat} = \frac{Q \times L}{A \times (L+P)} \quad \textbf{3-1}$$

Where, K_{sat} = saturated hydraulic conductivity (mm/s)
Q = flow through the saturated substrate (mm^3/s)
L = depth of substrate (mm)
A = cross-sectional area of substrate (mm^2)
P = water depth overlaying the substrate (head) (mm)

3.2.3.4 Substrate porosity

Porosity was measured using the saturation method (n = 10 measurements per substrate); a 100mL graduated cylinder was filled with substrate and weighed, after which water was added to the point where all voids were filled (saturated) and weighed again. From these results, porosity was calculated as a percentage using equation 3-2.

$$\emptyset = \left(\frac{Vv}{Vt}\right) \times 100 \quad \textbf{3-2}$$

Where, $\emptyset$ = porosity (%)
Vv = volume of void space (cm^3)
Vt = total volume of substrate and water (cm^3)

3.2.3.5 Scanning electron microscope

Images of the surfaces of the recycled glass, mussel shell, and gravel substrates were taken using a scanning electron microscope (SEM), model JEOL JSM-IT300 at the University of Canterbury. This allowed highly-magnified images of the surface of the substrate to be produced. SEMs work by scanning a focused high-energy beam of electrons along the surface of the sample. Interactions between the electrons and the atoms within the sample cause the atoms to become excited and emit secondary electrons, which are detected by the microscope to form an image. For the electrons to be absorbed and emitted, the sample must be conductive. None of the substrates were considered conductive, therefore, prior to using the microscope the substrates were coated with chromium and carbon. Images in this thesis were taken under vacuum conditions.

Energy-Dispersive X-Ray Spectroscopy

Energy-dispersive x-ray spectroscopy (EDS) analyses were conducted to determine the chemical characteristics of the recycled glass, mussel shell, and gravel. Using the fundamental principle that each element has a unique structure, EDS produces a unique set of peaks (showing elemental composition) depending on the number and intensity of the x-rays emitted from the specimen. The SEM at the University of Canterbury utilised model Oxford 50 mm^2 SDD detector with Aztec software for EDS analyses.

3.3 Laboratory Experiments

3.3.1 Synthetic Stormwater

Experiments were conducted using a synthesized stormwater, rather than natural stormwater. Given the focus of these experiments was on the three most common heavy metals found in stormwater (i.e. zinc, copper, and lead) it was deemed unnecessary to use natural stormwater given its complexity and variability. Using a synthetic stormwater allowed for more consistency and repeatability of the composition of the stormwater.

Stock solutions for zinc, copper, and lead were made up in the laboratory using laboratory quality chemicals. The chemicals were individually combined with 1 L of DI water in a laboratory-certified clean jar, creating a solution with a known quantity of contaminant. A 4 L batch of synthetic stormwater was made up as required at the beginning of each round of experiments, using the heavy metal stock solutions and DI water. Nutrients were also added to the synthetic stormwater to ensure the growth and survival of biofilm during the experiments. The batch was then split into four 1L glass jars (solution reservoirs), which had been laboratory cleaned to prevent contamination (see section 3.5.4 for equipment decontamination procedure). Each jar had contaminant and nutrient concentrations at levels

approximate to those shown in Table 3-2; these concentrations were chosen as typical concentrations of constituents found in natural stormwater. Additionally, dissolved organic carbon (DOC) was added to the solution as a carbon source for biofilm growth.

Table 3-2. Target concentrations of key dissolved heavy metals and nutrients in the synthetic stormwater and their chemical sources (modified from Seelsaen et al., 2006; Blecken et al., 2010).

Constituent	Mean concentration	Source
Zinc	280.2 µg/L	Zinc chloride ($ZnCl_2$)
Copper	154.0 µg/L	Copper chloride ($CuCl_2$)
Lead	41.8 µg/L	Lead acetate ($Pb(CH_3COO)_2$)
Dissolved NO_x-N	0.4 mg/L	Potassium nitrate (KNO_3)
Dissolved NH_4-N	0.22 mg/L	Ammonium chloride (NH_4Cl)
Organic N	5 mg/L	Nicotinic acid ($C_6H_5O_2N$)
Total Phosphorus	0.31 mg/L	Potassium dihydrogen phosphate (KH_2PO_4)
Dissolved Organic Carbon	10 mg/L	Sodium acetate (CH_3COONa)

3.3.2 Column Setup and Design

In total eight mesocosm-scale columns were constructed in the laboratory for use in all experiments. The columns were constructed using 1000mL Polymethylpentene (PMP) plastic measuring cylinders, with an internal diameter of 42.33 mm. To allow the inflow of solution, each column had a tube connector fixed into the base and sealed with silicon. An outflow pipe was fitted at the 1000 mL level, and attached using a plastic connector that was held in place using glue and sealed with silicon. A diagrammatic representation of the water flow through the systems is shown in Figure 3-4.

Once constructed the columns were decontaminated and experiments initiated. Two peristaltic pumps were used for both phases of experiments (one pump is limited to four pump heads and thus four columns). To ensure flow rates were consistent between the two pumps, inflow measurements were taken prior to beginning the experiments and checked periodically thereafter to maintain consistency. Solution, from the solution reservoir, was piped through size 6 polyurethane tubing, via the peristaltic pump that used Masterflex tubing, size 16. Columns were arranged on clamp stands to allow 'treated' solution to drain back into the solution reservoir by gravity, eliminating the need for further pumps.

A control experiment was conducted without any substrate in the columns to ensure the columns themselves would not contribute to the removal of heavy metals; in all cases, heavy metal removal was negligible.

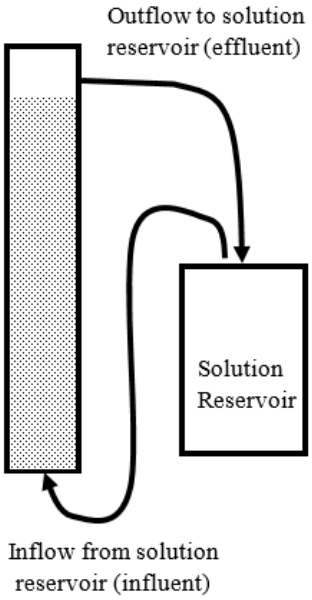

Figure 3-4. A diagrammatic representation of the column setup for experiments. Each column had its own synthetic stormwater solution reservoir, with influent inflow at the bottom and effluent outflow at 1000 mL.

3.3.3. Phase One – Layered Biofilm Experiments

Phase one experiments were designed to achieve three objectives. Firstly, to determine if biofilm grown under different light conditions showed differing removal rates of dissolved heavy metals from a synthetic stormwater. Secondly, to quantify how effectively biofilms adsorbed dissolved heavy metals from stormwater over time, and lastly, to assess the efficiency of two alternative substrates to remove dissolved heavy metals from a synthetic stormwater.

3.3.3.1 Column composition and operation

Columns were constructed as described in section 3.3.2 and arranged along a workbench such that all eight columns could be accessed at one time. The substrate was added to the columns to a total depth of 300 mm, according to the specific treatment conditions shown in Table 3-3. Individual experiments were carried out for each biofilm treatment (covered or uncovered), experiments using covered biofilm were covered to prevent light exposure. Each treatment (control and both biofilm treatments) occurred in quadruplicate. Substrate depth was similar to that recommended by the Christchurch City Council rain garden design construction and maintenance manual (CCC, 2016) which specifies a minimum media depth of 300 mm. Treatment experiments had biofilm added to the columns in three layers of 5 mm each, separated by layers of clean substrate (Figure 3-5 A). Each column had its own 1L solution reservoir, as depicted in Figure 3-4 and Figure 3-5 B. A batch of synthetic stormwater was made up as needed at the beginning of the experiments (described in section 3.3.1) and divided evenly into the 1L

solution reservoirs of each column. At the commencement of the experiment the synthetic stormwater was pumped, using a peristaltic pump, through the inflow valve at the bottom of the column and 'treated' effluent was drained back into the solution reservoir from which it originated, thus circulating the synthetic stormwater.

Table 3-3. Substrate compositions within constructed columns.

System treatment	Depth of substrate in column (mm)			Total depth of substrate (mm)
	Recycled glass	Mussel shell	Biofilm [1, 2]	
Control 1	300	-	-	300
Control 2	-	300	-	300
Treatment 1	285	-	15	300
Treatment 2	-	285	15	300

Note: 1. Biofilm was sourced from either covered or uncovered stock biofilm.
2. Biofilm occurred in three 5mm layers, total biofilm depth was 15 mm.

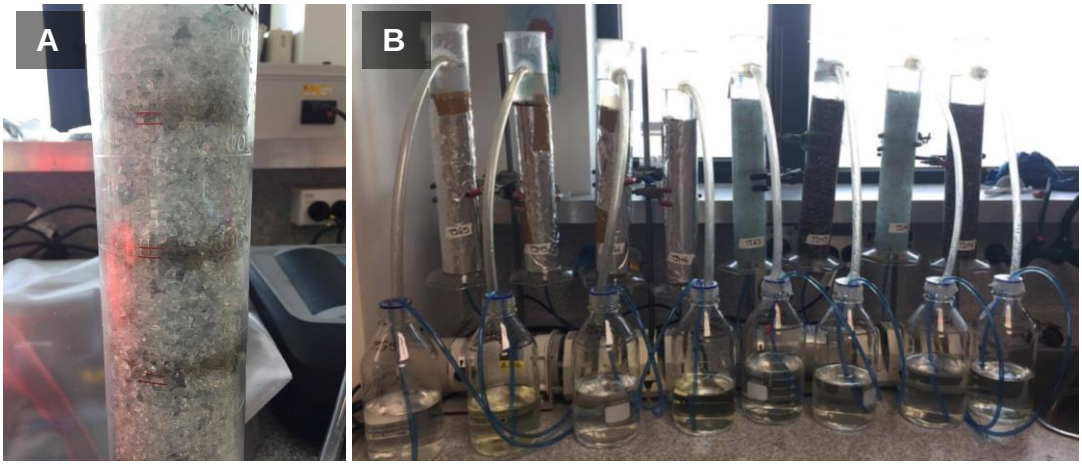

Figure 3-5. Phase one experiment setup. (A) Image shows the three layers (approximately 5 mm each) of uncovered biofilm in a column prior to the start of experiments. (B) Image shows covered experiments on the left and uncovered experiments on the right.

3.3.3.2 Sampling and solution characterisation

Samples of the synthetic stormwater influent were taken from each solution reservoir immediately prior to the start of the experiments, and at the same time, pH and specific conductance were measured. Initial total alkalinity was measured at this time also. After the experiments began, samples and solution characterisation occurred every 24 hours for a total of 168 hours (7 days) from each respective solution reservoir (see section 3.5.1 for sampling collection details). At the end of the experiment samples of each solution were kept for total alkalinity testing, which occurred within 24 hours of the end of the experiment. All samples of solution were kept in labelled ICP MS tubes, preserved and remained

refrigerated until a time when they were taken to determine dissolved heavy metal concentrations by ICP MS (see section 3.5.2 for more detail on solution characterisation and heavy metal analysis).

3.3.4 Phase Two – Whole Column Biofilm Experiments

Phase two experiments focused on dense autotrophic biofilm (uncovered biofilm) and its ability to adsorb dissolved heavy metals (zinc, copper, and lead) from a synthetic stormwater. Columns were arranged on the benchtop and dense biofilm was grown in the columns over a 6 week period, after which experiments were conducted.

3.3.4.1 Column composition and operation

Using the same columns as phase one (construction described in section 3.3.2), four columns were filled with 300 mm of each substrate, i.e. recycled glass and mussel shell. Three columns of each substrate were used to grow the biofilm; while the remaining column for each substrate was a control (no biofilm) (Figure 3-6). To facilitate biofilm growth within the columns, water from Okeover Stream was collected and circulated through the columns; nutrients were added to this solution to further facilitate the growth of biofilm, in concentrations described in section 3.1.1, Table 3-1. Once biofilm growth became established, water from Okeover Stream was substituted for DI water with added nutrients; this allowed the columns to be flushed of potential contaminants before beginning the experiments while providing nutrients to continue biofilm growth. Experiments commenced when biofilm growth was considered established; this stage was determined visually based on biofilm growth throughout each column (Figure 3-7). Once established, experiments were run in the same manner as phase one. Such that, each column had its own 1L solution reservoir and a batch of synthetic stormwater was made up as needed at the beginning of the experiments (described in section 3.3.1) and split evenly between the 1L solution reservoirs of each column. At the commencement of the experiment the stormwater was pumped, using a peristaltic pump, through the inflow valve at the bottom of the column and 'treated' effluent was drained back, by gravity, into the solution reservoir from which it originated, thus circulating the synthetic stormwater.

3.3.4.2 Sampling and solution characterisation

Samples of the synthetic stormwater influent were taken from each solution reservoir immediately prior to the start of the experiments, and at the same time, pH and specific conductance were measured. Initial total alkalinity was measured at this time also. Results from phase one experiments revealed that the greatest pollutant removal occurred within the first 24 hours, which prompted a change in the sampling regime for phase two. Samples and solution characterisation occurred every 2 hours for the first 6 hours, then 6 hours after that (12 hours after the beginning of the experiment) and finally 24 and 48 hours after the start of the experiments. The experiments ended after 48 hours (2 days). Solution samples and characterisation were taken from the respective solution reservoir (see section 3.5.1 for sampling

collection details). At the end of the experiment samples of each solution reservoir were kept for total alkalinity testing, which occurred within 24 hours of the end of the experiments. All solution samples were kept in labelled ICP MS tubes, preserved and remained refrigerated until a time when they were taken to determine dissolved heavy metal concentrations by ICP MS (see section 3.5.2 for more detail on solution characterisation and heavy metal analysis).

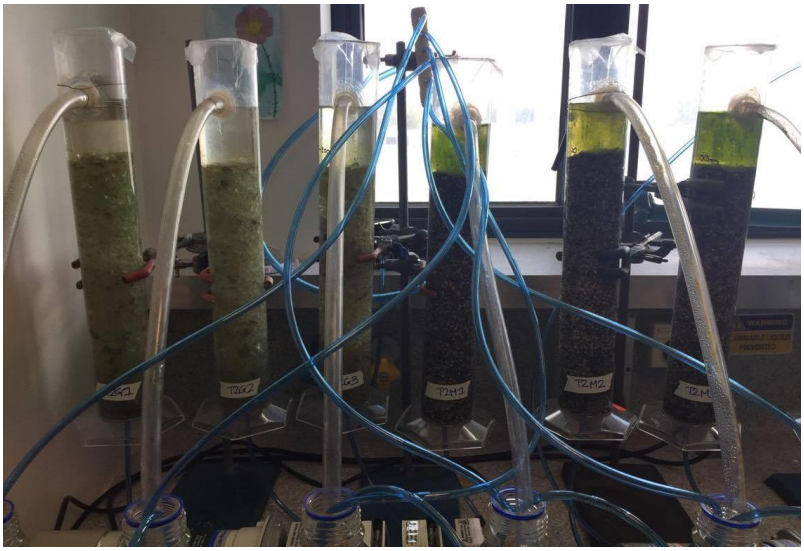

Figure 3-6. Phase two experiment setup. Three columns contained recycled glass (left) and three contained mussel shell (right). Control columns were also constructed but are not pictured.

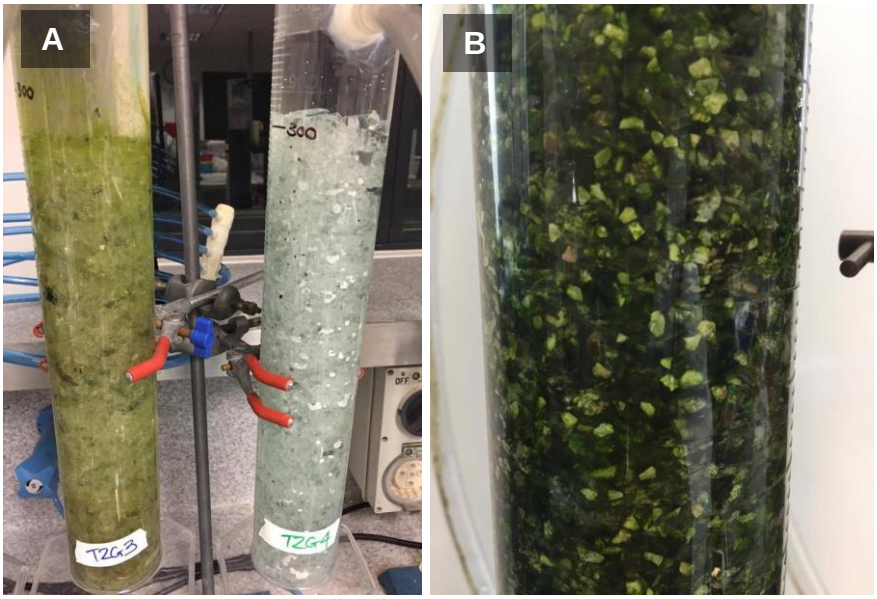

Figure 3-7. Phase two biofilm growth. (A) Comparison between a column with 6 weeks of biofilm growth (left) and the control column. (B) Dense biofilm growth can be seen in the mussel shell columns after 6 weeks.

3.4 Additional Experiments

3.4.1 Gravel Experiments

Gravel was used as a third substrate to provide an alternative control given that the original control (recycled glass) showed heavy metal removal abilities. Grade 5 chip (5 – 8 mm) was obtained from Park House Garden Supplies (Figure 3-8); grade 5 was the smallest size chip available for purchase and was chosen to best match the particulate size of the recycled glass and mussel shell. The gravel was washed and dried before being used in the experiments. No biofilms were grown on the gravel for these experiments due to time restraints, however, experiments were conducted in an identical manner to the whole column biofilm experiments (see section 3.3.4 for details on column setup and sampling, and characterisation). Samples were taken for analysis by ICP MS for dissolved heavy metals.

Figure 3-8. Gravel substrate used as an alternative control.

3.4.2 Batch Adsorption Experiment

Batch adsorption experiments were conducted to confirm the capacity of the gravel, recycled glass, and mussel shell to adsorb the dissolved heavy metals. The batch experiments were run in 2L beakers with the equivalent of 300 mm of substrate, in a PMP column, added to the beaker after which 1 L of synthetic stormwater was added (section 3.3.1). The contents of the beaker were stirred and left, covered, on the bench for the duration of the experiment (Figure 3-9). Discrete samples of the synthetic stormwater were taken directly from the beaker at 2, 4, 6, 12, 24, and 48 hours, as described in section 3.5.1. These samples were taken for analysis by ICP MS for dissolved heavy metal concentrations. pH was also measured at these sampling times.

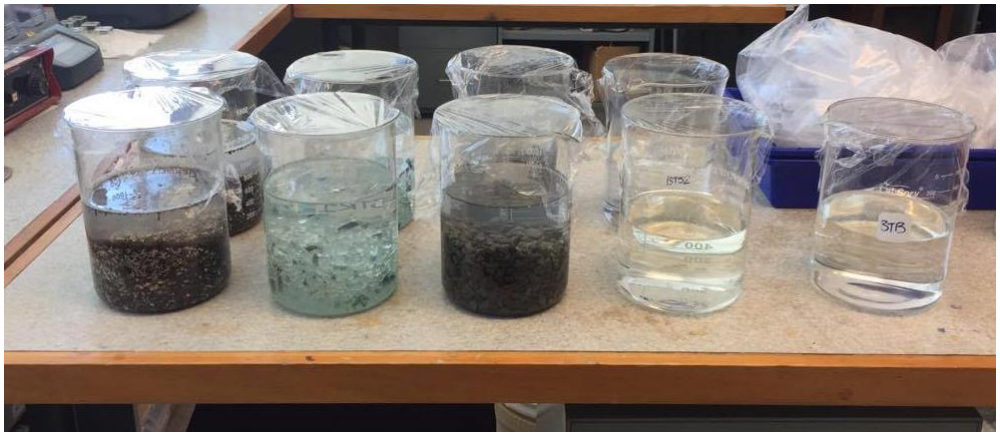

Figure 3-9. Batch adsorption experiments. Image shows the substrates (from left to right) mussel shell, recycled glass, gravel, blank synthetic stormwater, and DI water blank.

3.5 Sampling and Analytical Methods

3.5.1 Sample Collection

Influent synthetic stormwater samples were taken from each solution reservoir immediately prior to the commencement of the experiments, after which samples of effluent were taken at each sampling instance from the respective solution reservoir. Discrete samples of 15 mL were manually taken at each sampling instance (initial influent and effluent); all samples were taken directly from the respective column solution reservoir. Furthermore, at each sampling instance, DI water was sampled as a blank; this sample was taken for quality assurance/control purposes. Additionally duplicate and triplicate samples were taken every 10 and 20 samples respectively (section 3.5.3). Samples were collected using a 10mL pipette and laboratory-cleaned pipette tips. Effluent characterisation (pH, specific conductance, and temperature) was recorded at the same time as samples were taken for both phases of experiments.

3.5.2 Synthetic Stormwater Characterisation

Sampling and characterisation of the synthetic stormwater took place at each sampling time, except total alkalinity, which was tested at the beginning and end of an experimental run. Dissolved heavy metals (section 3.5.2.3) were determined in phase one, phase two, gravel, and batch adsorption experiments, also biofilm grown in phase two experiments was digested to determine the concentration of heavy metals within the biofilm itself (section 3.5.2.4).

3.5.2.1 pH and specific conductance

Samples of synthetic stormwater were taken from the solution reservoir for pH analysis at the same time as sample collection. Sample pH was measured using a calibrated EDT pH meter. Specific

conductance and temperature were measured directly from the solution reservoir using a calibrated YSI 30 specific conductance and temperature meter. Specific conductance was internally calculated within the meter from electrical conductivity normalized to 25°C using equation 3-3.

$$\mathrm{SC} = \frac{Conductivity}{(1+TC \times (T-25))} \quad \textbf{3-3}$$

Where, SC = specific conductance (25°C)
TC = constant (0.0191)
T = temperature (°C)

3.5.2.2 Total alkalinity

Total alkalinity was measured at the beginning and end of each experimental run. At the beginning, total alkalinity was tested once from the 4 L batch after it had been split into the 1L solution reservoirs. At the end of each experiment, samples from each column solution reservoir were taken to determine total alkalinity. Where possible a sample of 200 mL of the synthetic stormwater was taken and total alkalinity was determined using the titration method, method APAH 2320 B (APHA, 2005). Samples were titrated with 0.1 N hydrochloric acid (HCL) until a pH of 4.5 was reached, where the pH was measured using a calibrated EDT meter and the total titrant was recorded. Total alkalinity was calculated using equation 3-4. All total alkalinity analyses were completed within 24 hours of sampling.

$$\mathrm{Alk}_t = \frac{A \times N \times 50{,}000}{mL\ sample} \quad \textbf{3-4}$$

Where, Alk_t = total alkalinity (mg/L as $CaCO_3$)
A = volume of titre
N = normality of HCL

3.5.2.3 Dissolved heavy metals

The focus of this research was on the removal of dissolved heavy metals, which were sampled following method 3030 B (APHA, 2005) as described below.

A 15 mL sample of the solution was taken from each solution reservoir at each sampling time and stored in labelled ICP MS tubes. The samples were preserved to a pH ≤ 2, using trace grade nitric acid (HNO_3) promptly after sampling. At the conclusion of each experiment, approximately 7 mL of the sample was

taken and filtered through a 0.45µm syringe filter and sent for ICP MS analysis. This left a further 7 mL of the sample in reserve. Between sampling occasions, samples were kept in a refrigerator.

All heavy metal samples were analysed for dissolved heavy metals using an Inductively Coupled Plasma-Mass Spectrometer (ICP MS) at the University of Canterbury's Chemistry Department, in accordance with method 3125 B (APHA, 2005).

3.5.2.4 Biofilm digestion

At the end of the whole column biofilm experiments (phase two), biofilm was removed from the recycled glass columns and digested (method 3030 E; APHA, 2005) to determine the concentration of heavy metals within the biofilm itself.

Recycled glass, with biofilm attached, was tipped into a beaker and washed with DI water. The biofilm was detached from the recycled glass by shaking and the columns were scraped to remove biofilm growth on the internal wall of the column. The liquid biofilm mixture was decanted into a 500mL measuring cylinder and left to settle for 24 hours (Figure 3-10). The settled biofilm was separated from the supernatant and added to a 500mL volumetric flask, which was made up to 500 mL using left over supernatant. Three 100 mL subsamples were transferred to Phillips beakers and 5 mL of concentrated HNO_3 was added. The samples were brought slowly to boiling point; to aid boiling and ensure the sample was boiled evenly, boiling chips were added. Boiling continued until the digestion was complete, indicated by a light coloured, clear solution. The digested solution was filtered into a volumetric flask and diluted to make a 100 mL solution. Portions of this solution were taken for total heavy metal analysis by ICP MS in accordance with method 3125 B (APHA, 2005).

Figure 3-10. Whole column biofilm digestion. Recycled glass was washed with DI water; the water was decanted into 50mL measuring cylinders (pictured) and left to settle.

3.5.3 Equipment Calibration and Quality Control

Water quality parameters (pH, specific conductance, and alkalinity) were measured using portable, calibrated instruments. Instruments were calibrated with fresh standards prior to use. The pH probe was calibrated daily, prior to use and periodically throughout the day if characterisation occurred at multiple times during the day. Calibration was done using fresh 4.0, 7.0, and 10.0 SU buffers. Specific conductance was calibrated using a standard of 0.01 M Potassium chloride (KCL) (1412 µS/cm at 25°C), at the beginning of each experimental run.

To ensure the accuracy and reliability of the results, blanks, duplicates, and triplicates were included during influent and effluent sampling. Blank samples consisted of DI water that was treated identically to the experimental samples; blank samples were taken at every sampling occasion. Duplicate and triplicates of the experimental samples were taken after every 10 and 20 sampling occasions respectively.

3.5.4 Equipment Decontamination

All equipment used at any stage of the experiments was decontaminated. Containers used during the experiments, for example, columns, solution reservoirs, and 4L mixing container were soaked in a 5% phosphoric solution for a period of 12 – 24 hours before use. This removed any residual metals from the containers. After soaking, the equipment was rinsed in tap water and then with DI water. Additionally, any other containers, piping, pipette tips, and stirring utensils were rinsed using the same 5% solution and washed thoroughly before use. Where possible metal equipment was avoided.

3.5.5 Data Analyses

Removal efficiency (equation 3-5) of each substrate was calculated for each heavy metal based on the concentrations measured in the synthetic stormwater influent and effluent. This allowed comparisons to be made between each substrate's ability to remove dissolved heavy metals. Removal efficiency was calculated as:

$$\text{Removal efficiency (\%)} = \frac{(C_i - C_e)}{C_i} \times 100 \qquad \textbf{3-5}$$

Where, C_i = concentration in synthetic stormwater influent (µg/L)
C_e = concentration in synthetic stormwater effluent (µg/L)

Normalised values (equation 3-6) were calculated from the concentrations measured in the synthetic stormwater influent and effluent. This allowed for easier comparison between treatment concentration reductions because often treatments had different influent concentrations. Normalised values were calculated as follows:

$$\text{Normalised value} = \frac{C}{C_o} \quad \textbf{3-6}$$

Where, C = concentration in synthetic stormwater effluent at any time (µg/L)
C_o = concentration in synthetic stormwater influent (µg/L)

Regression analyses were conducted on the batch adsorption experiments to explore the relationship between pH and the removal efficiency for each substrate. The regression analyses were completed in Excel, using the data analysis tool.

Chapter 4. Results

This chapter describes the results of biofilm and substrate characterisation, as well as phase one, phase two, gravel, and the batch adsorption experiments (henceforth referred to as adsorption experiments). Images of the biofilm are displayed in section 4.1, followed by the results of the substrate characterisation (section 4.2), scanning electron microscope (section 4.2.1), and energy-dispersive x-ray spectrometry (section 4.2.2). Additionally, the synthetic stormwater characterisation is presented in section 4.3. Section 4.4 and section 4.5 correspond to layered biofilm (phase one) and whole column biofilm (phase two) experiments respectively and are organised such that contaminants (zinc, copper, and lead) are discussed individually. Lastly, results of the gravel (section 4.6), and the adsorption experiments (section 4.7) are presented. Full EDS reports, water quality data sets, effluent metal concentrations (for gravel and adsorption experiments only), and removal efficiency data sets are presented in Appendices A, B, C, and D respectively.

4.1 Biofilm Characterisation – Microscope Images

Microscope images revealed a biofilm with a diverse composition. The biofilm contained predominantly freshwater microorganisms, which was unsurprising given that the biofilm was seeded with water from the freshwater environments of Okeover and Haytons Streams. Dominant groups included blue-green algae (Cyanobacteria), green algae (Chlorophyta), and diatoms (Bacillariophyceae). Furthermore, microscopic animals can be seen (Figure 4-1), most commonly Rotifers (Rotifera) and ciliate protozoa (Ciliophora). Bacteria were also present, however, due to limitations in magnification and imaging, identification was not possible.

Table 4-1 lists the most common genuses of freshwater microorganisms seen within the biofilm under their common names (and phylum). Unfortunately, diatoms are difficult to identify beyond the phylum level and are thus not included despite being present in large numbers, as can be seen in Figure 4-2 F as brown organisms within the biofilm matrix. Blue-green algae (Figure 4-2 A and F) were predominantly present as undifferentiated filaments, for example, within Figure 4-2 F a ‘stringy’ type filament is observed in the background identified as cf. *Leptolyngbya*. Green algae are a diverse group of single cell or unicellular organisms (Figure 4-2 B – E); an exception to this is the filamentous green algae in Figure 4-2 B.

Table 4-1. Dominant groups of freshwater microorganisms, identified within the biofilm, listed under their common names (and phylum).

Blue-green algae (Cyanobacteria)	Green algae (Chlorophyta)
Leptolyngbya	*Chlorella*
Phormidium	*Chlorosarcina*
-	*Gloeocystis*
-	*Klebsormidium*
-	*Scenedesmus*
-	*Ulothrix*

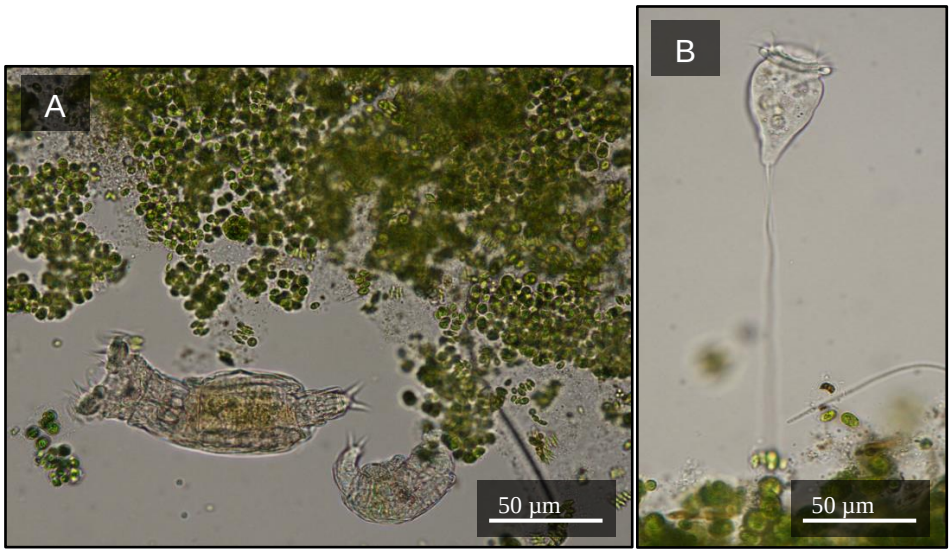

Figure 4-1. Images of the biofilm taken under the microscope containing microscopic animals. (A) Phylum Rotifera; (B) Genus *Vorticella* from the Phylum Ciliophora.

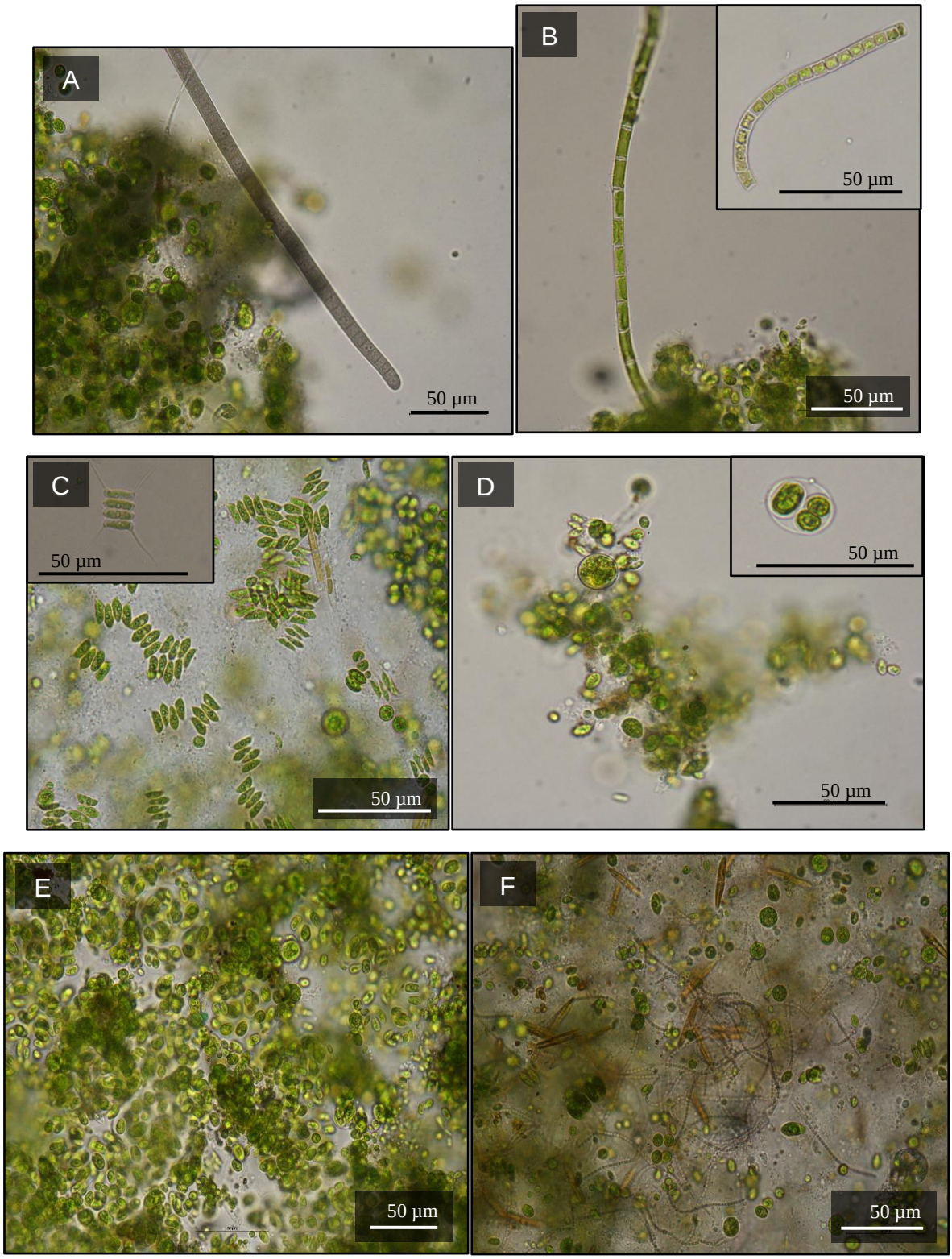

Figure 4-2. Microscope images of the biofilm. (A) *B*lue-green algae *Phormidium*; (B) cf. *Klebsormidium,* insert shows cf. *Ulothrix;* (C) Prevalent green algae *Scenedesmus*; (D) Diverse unicellular green algae; (E) Overview of biofilm, showing diverse unicellular green algae; (F) Diatoms with the cyanobacteria cf. *Leptolyngbya* and diverse unicellular green algae.

4.2 Substrate Characterisation

Results from the characterisation of the recycled glass and mussel shell are shown in Table 4-2. Both substrates had low concentrations of background heavy metals, meaning they were unlikely to influence the effluent concentration of dissolved heavy metals. The pH measured for both substrates was identical and highly alkaline. Additionally, very high hydraulic conductivity values were measured for both substrates, but more so for the recycled glass than the mussel shell. This is likely to be the result of the highly angular nature of the recycled glass. Despite this the mussel shell displayed a greater porosity than the recycled glass.

Table 4-2. Results from the substrate characterisation.

Substrate	**Character**					
	Average dissolved metal concentrations (mg/kg)			pH	Hydraulic conductivity (m/hr)	Porosity (%)
	Zinc	Copper	Lead			
Recycled glass	1.095	0.529	0.064	9.3 ± 0.1	37.4 ± 5.0	41.5 ± 1.2
Mussel shell	1.201	1.507	0.068	9.3 ± 0.2	21.5 ± 3.2	55.5 ± 0.8

4.2.1 Scanning Electron Imaging

The images taken using the scanning electron microscope (SEM) clearly showed the structure and surface of the recycled glass (Figure 4-3), mussel shell (Figure 4-4), and gravel (Figure 4-5).

The surface of the recycled glass was smooth and angular. At higher magnifications it was clear that there were particulates on the surface, and in some cases, there were engrained striations. The cause of these striations is likely a result of manufacturing. The mussel shell, on the other hand, showed a very porous surface, which may be beneficial for the removal of heavy metals; furthermore, particulates were present on the surface of the mussel shell. Gravel had a similarly porous surface texture to the mussel shell and had particulates present on the surface.

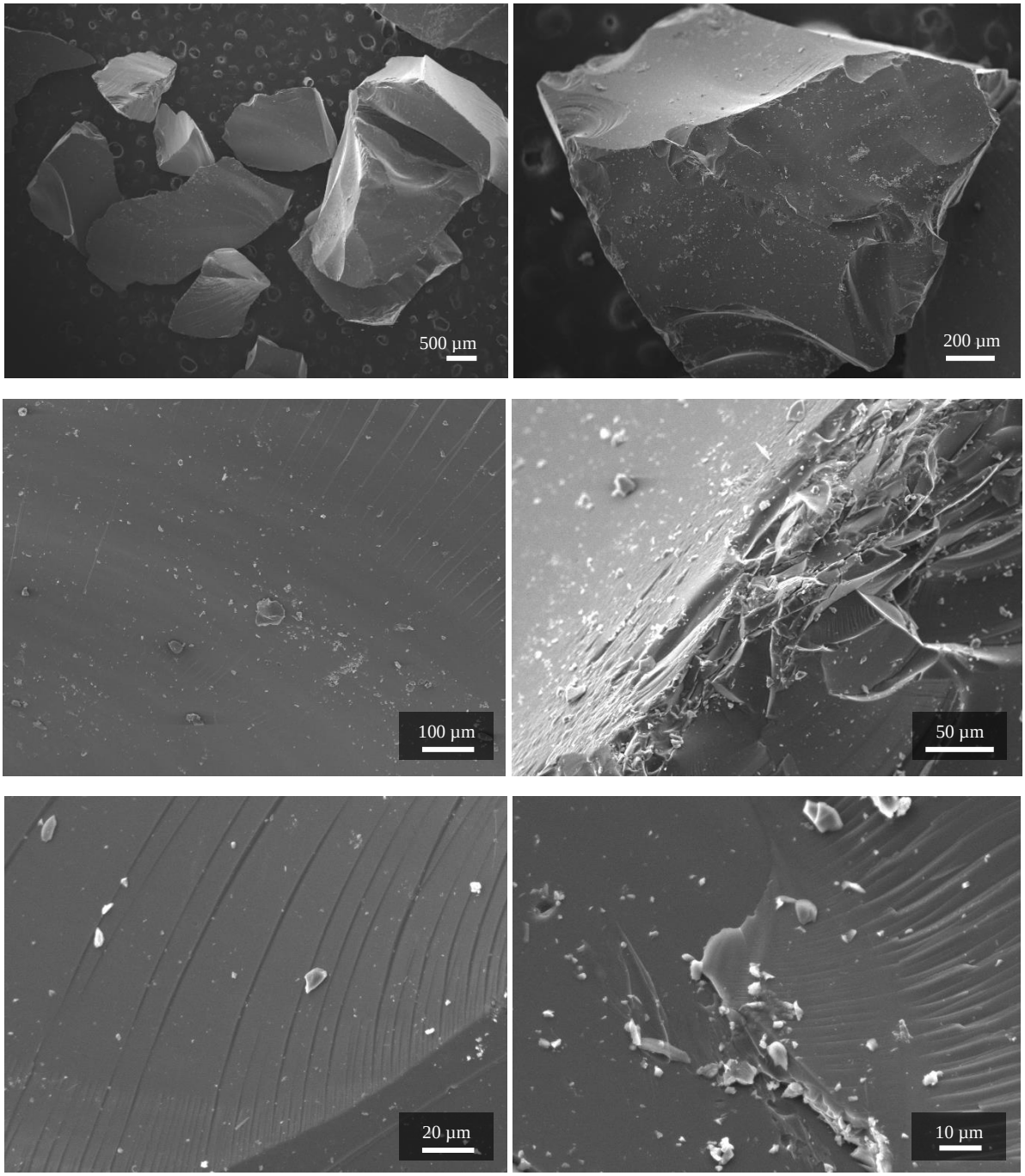

Figure 4-3. Scanning electron microscope images of the recycled glass substrate at various magnifications.

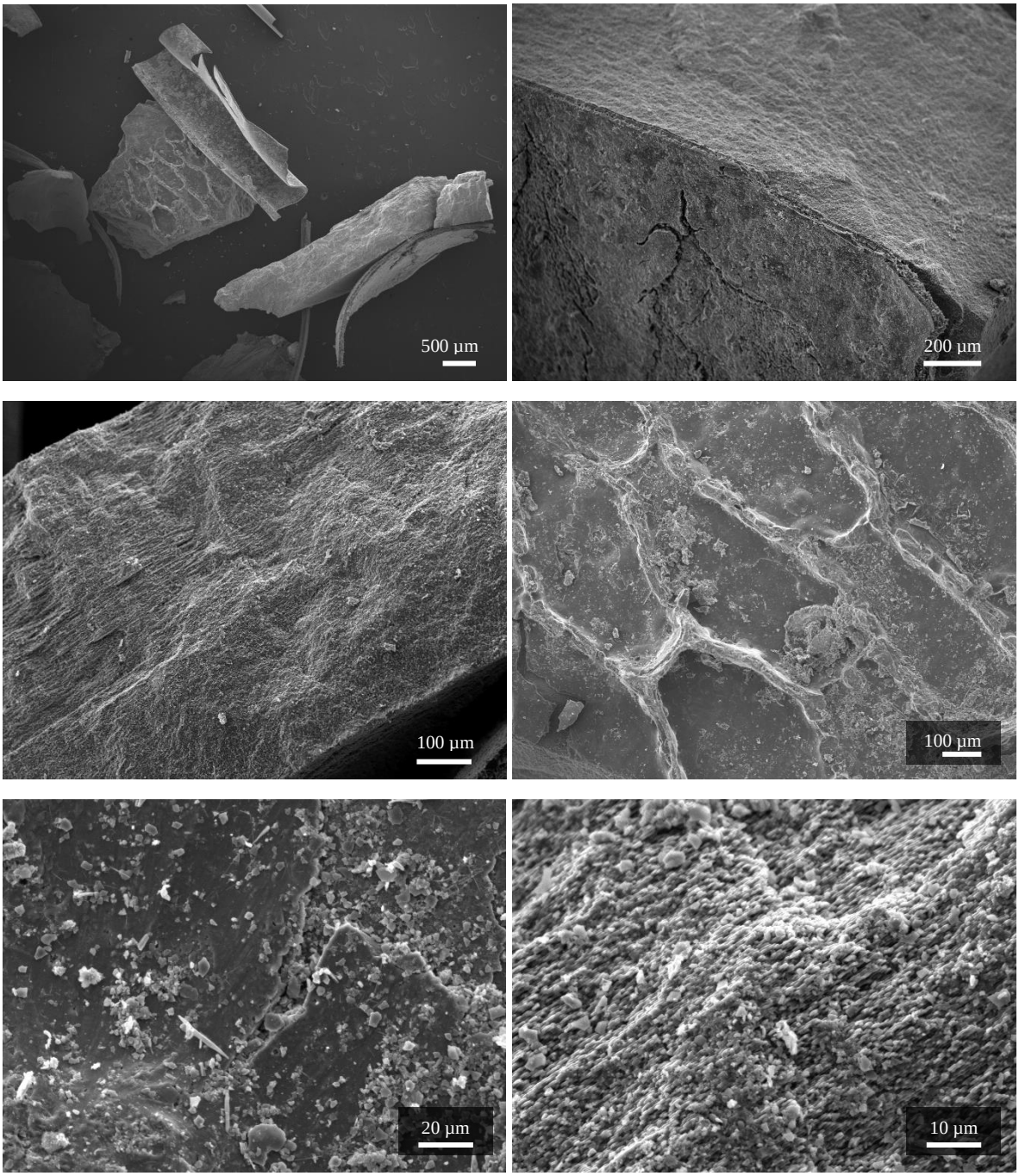

Figure 4-4. Scanning electron microscope images of the mussel shell substrate at various magnifications.

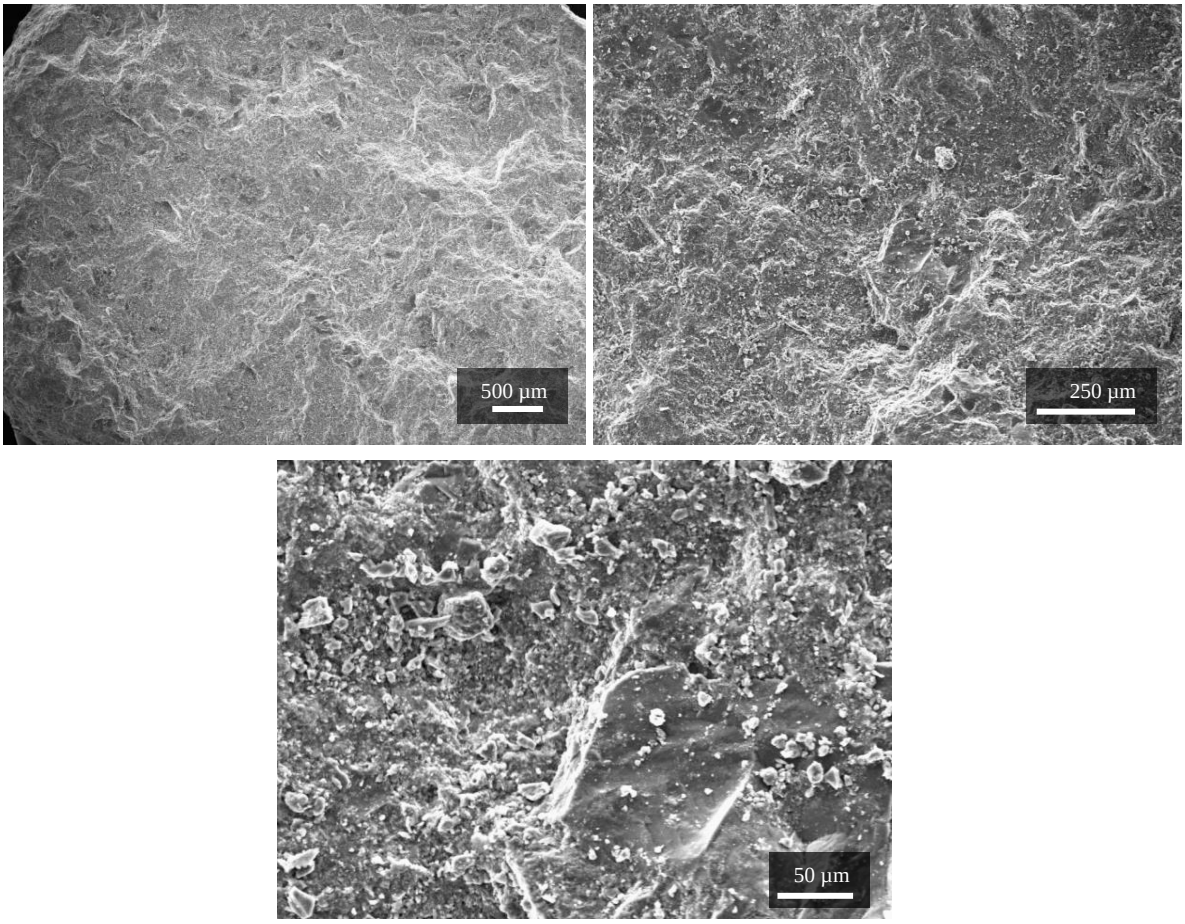

Figure 4-5. Scanning electron microscope images of the gravel substrate at various magnifications.

4.2.2 Energy-Dispersive X-Ray Spectroscopy

Results of the energy-dispersive x-ray spectroscopy (EDS; Table 4-3) show the chemical characteristics (mean ± SD Wt%) of the recycled glass, mussel shell, and gravel. Full EDS reports can be found in Appendix A.

Table 4-3. Individual chemical characteristics (mean ± SD Wt%) of the recycled glass (n = 6), mussel shell (n = 3), and gravel (n = 3).

Recycled glass		**Mussel shell**		**Gravel**	
Element	Wt%	Element	Wt%	Element	Wt%
Oxygen	45.8 ± 0.7	Oxygen	69.4 ± 1.6	Oxygen	47.2 ± 1.2
Silicon	33.6 ±0.7	Calcium	21.7 ± 0.7	Silicon	39.7 ± 3.3
Sodium	10.9 ± 0.3	Sulfur	3.7 ± 0.4	Aluminium	5.4 ± 2.3
Calcium	5.3 ± 0.2	Aluminium	1.7 ± 0.3	Potassium	2.6 ± 2.3
Magnesium	3.3 ± 0.4	Silicon	1.7 ± 0.3	Iron	2.4 ± 0.0

4.3 Synthetic Stormwater Characterisation

Influent (hour = 0) synthetic stormwater characterisation and dissolved heavy metal concentrations are reported in Table 4-4 and Table 4-5 respectively. The synthetic stormwater was made from deionized water mixed with various constituents to create a composition similar to that of natural stormwater.

Both the specific conductance and alkalinity fall within levels recorded for natural stormwater (e.g. Datry et al., 2003). Furthermore, the pH recorded is slightly more acidic than reported for natural stormwater (e.g. Mosley et al., 2001) but is typical of the pH reported for precipitation (e.g. Pennington et al., 2008). Typical dissolved heavy metal concentrations were achieved in the synthetic stormwater.

Table 4-4. Influent (hour = 0) synthetic stormwater characterisation for pH, specific conductance, and alkalinity. Values represent mean ± standard deviation.

	Character		
	pH	Specific conductance (µS/cm)	Alkalinity (mg/L as $CaCO_3$)
Synthetic stormwater	5.5 ± 0.5	57.6 ± 47.9	14.7 ± 10.9

Table 4-5. Influent (hour = 0) dissolved heavy metal concentrations in the synthetic stormwater and the target concentrations. Values represent mean ± standard deviation.

Dissolved heavy metal	Synthetic stormwater (µg/L)	Target concentration (µg/L)
Zinc	284.6 ± 17.9	280.2
Copper	109.4 ± 6.1	154.0
Lead	32.8 ± 3.1	41.8

4.4 Phase One – Layered Biofilm Experiments

4.4.1 Effluent Characterisation

Water quality parameters, pH, specific conductance, and alkalinity, were measured throughout the experiments. The pH and specific conductance were measured initially and then every 24 hours after the beginning of the experiment, for a total of 7 days (168 hours). Total alkalinity was measured at the beginning (influent; hour = 0) and end of the experiments (hour = 168). See Table 4-4 and Table 4-5 for influent synthetic stormwater characterisation results. A summary of the mean ± standard deviation (SD) pH, specific conductance, and total alkalinity are shown in Table 4-6 (see Appendix B for full set of water quality results).

Effluent pH was averaged over the duration of the experiments for both the recycled glass (n = 83 measurements) and mussel shell (n = 83 measurements) substrates. Both substrates achieved a slightly alkaline pH within the first 24 hours of the start of the experiment (influent pH = 5.5 ± 0.5), and remained stable, for both substrates, for the remainder of the experiment.

Specific conductance for both substrates was elevated compared to the influent (influent specific conductance = 57.6 ± 47.9 µS/cm) for all experiments. This trend was amplified in the mussel shell columns, with notable increases in specific conductance seen after 24 hours (343.6 ± 120.0 µS/cm; n = 12 measurements) and again after 168 hours (560.9 ± 197.7 µS/cm; n = 12 measurements). This increase is likely due to the release of ions from the mussel shell. The recycled glass, on the other hand, demonstrated a much less notable change in specific conductance after 24 hours (108.0 ± 32.1 µS/cm; n = 12 measurements) and only a slight increase at 168 hours (126.0 ± 32.3 µS/cm; n = 12 measurements).

Total alkalinity increased in both substrates (influent alkalinity = 14.7 ± 10.9 mg/L as $CaCO_3$) where mussel shell (n = 8 measurements) showed the greatest increase in alkalinity (184.3 ± 50.3 mg/L as $CaCO_3$) compared to the recycled glass (57.6 ± 24.6 mg/L as $CaCO_3$) after 168 hours (n = 10 measurements).

Table 4-6. Water quality parameters measured in treated effluent for the recycled glass and mussel shell substrates during phase one (combined results from all treatment conditions for each substrate).

Parameter	Mean value ± SD		
	Time measured (hours)	Recycled glass	Mussel shell
pH	24 – 168	7.8 ± 0.3	8.1 ± 0.1
Specific conductance (µS/cm)	24	108.0 ± 32.1	343.6 ± 120.0
	168	126.0 ± 32.3	560.9 ± 197.8
Alkalinity (mg/L as $CaCO_3$)	168	66.9 ± 14.3	184.3 ± 50.3

4.4.2 Effluent Metal Concentrations

All mean concentrations of dissolved zinc, copper, and lead were below their respective initial concentrations, indicating the pollutant removal capabilities of the systems. Removal efficiencies (%), calculated as the difference between contaminant loads in influent and effluent, were high for both substrates and all treatment conditions.

4.4.2.1 Zinc

Both substrates successfully treated dissolved zinc to varying levels (Table 4-7). Across all experimental runs initial zinc concentrations showed slight variation (range = 267.7 – 310.9 µg/L; target concentration = 280.2 µg/L). The biggest reduction in dissolved zinc occurred within the first 24 hours of the start of the experiments, which occurred for both substrates and all treatments.

Table 4-7. Dissolved zinc concentrations (µg/L; mean ± SD) in influent (hour = 0) and treated effluent for the recycled glass and mussel shell substrates with three treatment conditions. Treatment conditions are no biofilm (substrate only, control; n = 4), biofilm grown in dark conditions (covered biofilm; n = 4), and biofilm grown in light conditions (uncovered biofilm; n = 4).

Time (hours)	Recycled glass			Mussel shell		
	Control	Covered biofilm	Uncovered biofilm	Control	Covered biofilm	Uncovered biofilm
0	288.4 ± 0.4	272.3 ± 5.7	310.9 ± 13.4	291.8 ± 16.1	267.7 ± 4.2	283.9 ± 17.6
24	14.8 ± 7.9	70.4 ± 39.3	56.5 ± 11.4	9.2 ± 9.2	9.8 ± 8.8	5.4 ± 1.9
48	36.0 ± 7.9	54.3 ± 23.8	42.8 ± 4.3	5.2 ± 1.1	6.4 ± 3.7	7.4 ± 3.2
72	53.9 ± 13.3	50.2 ± 14.6	49.1 ± 7.8	7.9 ± 4.9[1]	3.4 ± 1.0	54.8 ± 84.7[1]
96	61.5 ± 4.8	48.9 ± 10.4	51.5 ± 6.5	4.2 ± 0.6	2.7 ± 0.3	33.0 ± 49.8[1]
120	64.6 ± 14.6	41.2 ± 3.0	26.8 ± 2.3	7.1 ± 4.7	2.7 ± 0.4	8.8 ± 5.5
144	63.8 ± 2.7	44.0 ± 9.2	35.9 ± 3.8	4.3 ± 4.7	2.8 ± 0.8	7.9 ± 3.4
168	63.2 ± 4.1	39.2 ± 8.4	42.7 ± 3.5	5.3 ± 1.1	2.8 ± 0.5	9.9 ± 4.9

Note: 1. Outlier present in data

All three recycled glass treatments showed a high level of dissolved zinc removal after 24 hours. After 24 hours the control treatment showed the highest reduction in dissolved zinc concentrations compared to the biofilm treatments, followed by the uncovered and then the covered biofilm treatment. From 72 hours onwards the uncovered biofilm treatment showed slightly better removal rates than either the control or covered biofilm treatments, however, the concentrations of dissolved zinc are practically the same in all three treatments. After this, some fluctuations in dissolved zinc concentrations can be seen in the biofilm treatments, however, both biofilm treatments consistently perform better than the control treatment. Overall, the uncovered biofilm treatment achieved the greatest reduction in dissolved zinc concentrations over time.

Mussel shell treatments showed a similar pattern with concentrations of dissolved zinc falling below 10 µg/L after just 24 hours. Similar concentrations of zinc were seen after the first 24 hours across the three mussel shell treatments. At 24 hours, the uncovered biofilm treatment showed slightly better removal of zinc; however, this changed towards the middle of the experiment (48 hours onwards) when the covered biofilm treatment showed lower levels of zinc in the effluent. From 24 to 168 hours, there were slight fluctuations in the concentration of zinc, along with two outliers, yet they remained very similar across all treatments and there was no further reduction seen.

Removal Efficiency

Both recycled glass (Figure 4-6 A) and mussel shell (Figure 4-6 B) substrates showed good removal efficiencies.

Recycled glass treatments with no biofilm (control) revealed an interesting trend. Initial removal, after just 24 hours, was high at approximately 95%, yet between 24 and 120 hours, the removal efficiency of the substrate decreased to approximately 77% and then levelled out for the remainder of the experiment. Both biofilm treatments displayed a different trend to the control in that the removal efficiency increased over the duration of the experiments. The uncovered biofilm treatment showed slightly higher removal efficiencies, with a range of 81 – 91%, when compared to the covered biofilm treatment (range = 74 – 85%) for the removal of dissolved zinc. However, in both biofilm treatments, after 72 hours removal rates were greater than that of the control treatment and continued on a positive trend until the end of the experiments at 168 hours.

Mussel shell columns displayed excellent dissolved zinc removal efficiencies, with treatment efficiencies all above 90%. Uncovered biofilm showed a slightly decreasing trend from 98 – 96% over the course of the experiment, however, there were some variations within this time. Both the control and covered biofilm treatments showed increased removal efficiencies over 168 hours. Covered biofilm showed the best removal of dissolved zinc, achieving a removal efficiency of 99% after 72 hours (range = 96 – 99% over 168 hours), and this rate of removal was maintained for the remainder of the experiment.

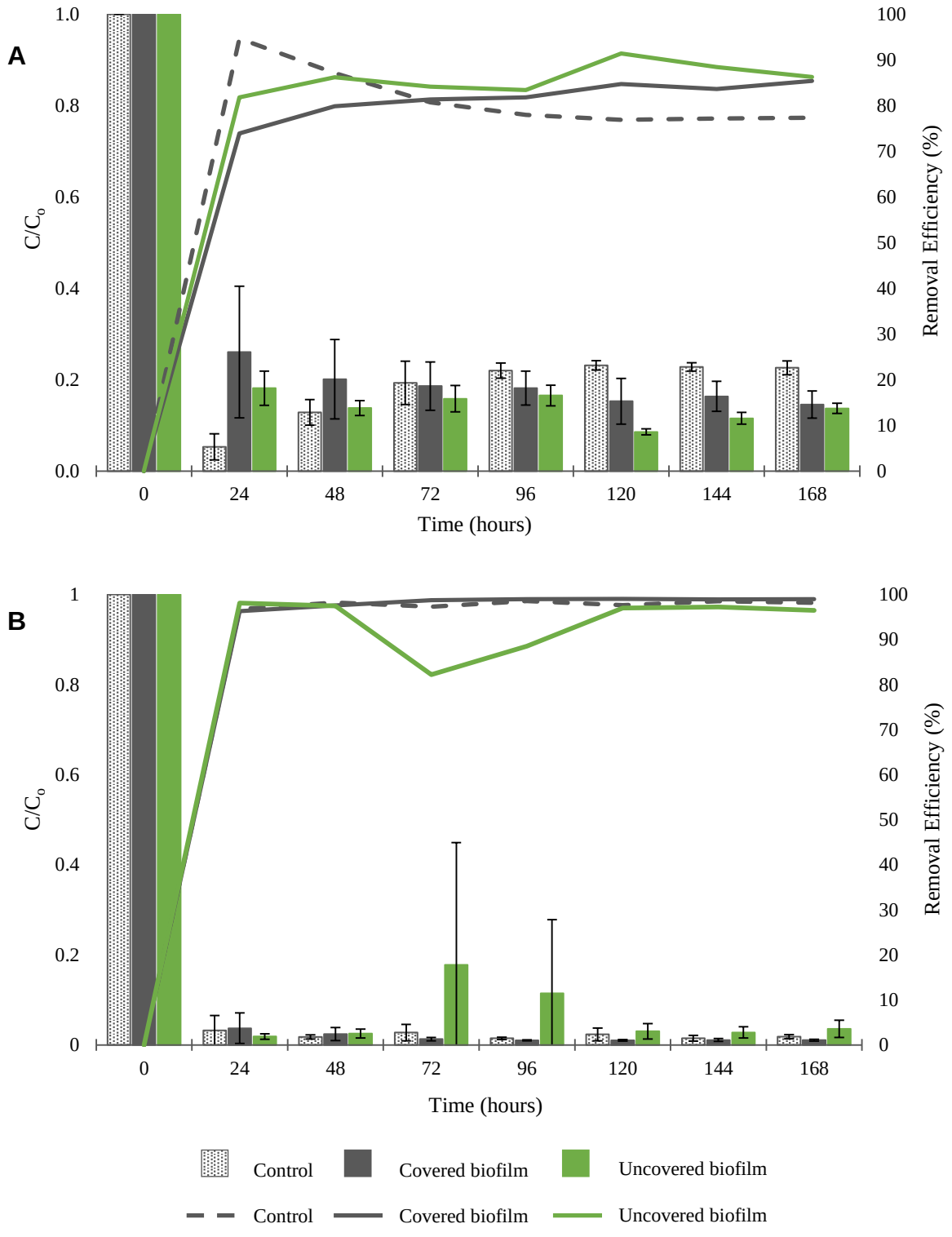

Figure 4-6. Normalised values of dissolved zinc (C/C_o, bars) in influent (hour = 0) and treated effluent; also, removal efficiency (%, lines) of dissolved zinc shown on the second axis. (A) Recycled glass and (B) mussel shell substrates with three different treatments; treatment 1 = control (no biofilm), treatment 2 = covered biofilm (biofilm grown in dark conditions), and treatment 3 = uncovered biofilm (biofilm grown in light conditions).

4.4.2.2 Copper

Copper was successfully treated in all experiments to varying levels (Table 4-8). Slight variations in influent copper concentrations can be seen across all experimental runs (range = 104.0 – 113.7 µg/L; target concentration = 154.0 µg/L). The biggest reduction in copper occurred within the first 24 hours of the beginning of the experiments, which occurred for both substrates and all treatments.

Table 4-8. Dissolved copper concentrations (µg/L; mean ± SD) in influent (hour = 0) and treated effluent for the recycled glass and mussel shell substrates with three treatment conditions. Treatment conditions are no biofilm (substrate only, control; n = 4), biofilm grown in dark conditions (covered biofilm; n = 4), and biofilm grown in light conditions (uncovered biofilm; n = 4).

Time (hours)	Recycled glass			Mussel shell		
	Control	Covered biofilm	Uncovered biofilm	Control	Covered biofilm	Uncovered biofilm
0	110.9 ± 5.1	106.7 ± 8.6	112.8 ± 6.9	113.7 ± 2.1	104.0 ± 3.4	105.1 ± 3.4
24	46.0 ± 9.0	43.4 ± 8.6	47.6 ± 9.9	25.2 ± 33.7[1]	7.9 ± 2.4	7.6 ± 3.5
48	44.4 ± 16.7	52.7 ± 9.6	46.6 ± 6.3	8.6 ± 5.6	7.2 ± 3.9	7.9 ± 3.7
72	50.3 ± 21.2	55.9 ± 8.9	47.2 ± 4.0	9.5 ± 5.9	6.6 ± 3.5	8.0 ± 3.4
96	50.9 ± 24.5	56.5 ± 5.5	50.5 ± 5.3	8.4 ± 4.7	6.4 ± 3.3	7.8 ± 2.8
120	57.7 ± 29.1	57.3 ± 3.0	52.8 ± 7.2	7.3 ± 4.4	5.6 ± 2.7	8.1 ± 2.6
144	57.0 ± 36.5	60.6 ± 3.8	54.9 ± 6.4	7.1 ± 4.3	5.3 ± 2.5	7.6 ± 7.9
168	58.6 ± 34.7	60.6 ± 6.2	48.7 ± 11.8	7.1 ± 3.9	5.5 ± 1.3	7.4 ± 1.7

Note: 1. Outlier present in data

The concentration of dissolved copper reduced by half after 24 hours in all three recycled glass treatments, and but did not decrease further for the remainder of the experiments. Instead the concentrations tended to increase in all three treatments but was most noticeable in the covered biofilm treatment which showed an increase in copper concentrations of approximately 17 µg/L. Throughout the experiments the control and uncovered biofilm treatments showed similar dissolved copper concentrations, except at 168 hours when a difference of approximately 10 µg/L was seen between the two treatments. The uncovered biofilm treatment typically achieved the lowest concentrations of dissolved copper over time.

Mussel shell treatments saw the most dramatic decrease in dissolved copper concentrations and within the first 48 hours, concentrations dropped below 10 µg/L. After 48 hours the concentrations of dissolved copper were very similar between all treatments and remained stable for the duration of the experiment.

Removal Efficiency

After 24 hours, the percentage removal of dissolved copper in all recycled glass treatments was similar (control = 59%, covered biofilm = 54%, and uncovered biofilm = 58%) and remained very similar for the remainder of the experiments with all treatments showing slight decreases in removal efficiency over time (Figure 4-7 A). The covered biofilm treatment achieved the worst removal efficiency with an average removal, over 168 hours, of 47% (range = 43 – 54%). Both the control and uncovered biofilm treatments displayed similar removal efficiencies over the course of the experiment. The removal efficiency of the control treatment showed some variation between samples, especially between 48 to 120 hours, but still showed a general decreasing trend in removal. The same can be seen for the uncovered biofilm treatment, and while they show a general decreasing removal efficiency there was a spike at 168 hours. Overall the uncovered biofilm displayed a marginally better removal efficiency of copper than either of the other treatments in recycled glass media.

Mussel shell treatments showed excellent dissolved copper removal efficiency, with all treatments achieving upwards of 90% removal after 48 hours exposure (Figure 4-7 B). Furthermore, all treatments maintained relatively stable removal efficiencies over the duration of the experiments, for example, the control treatment showed an increase of only 1% and the covered biofilm an increase of 2.5% over time. The uncovered biofilm treatment achieved the lowest removal efficiency out of the three treatments but still achieved an excellent removal efficiency. Overall, the covered biofilm treatment performed the best with regard to removal efficiency.

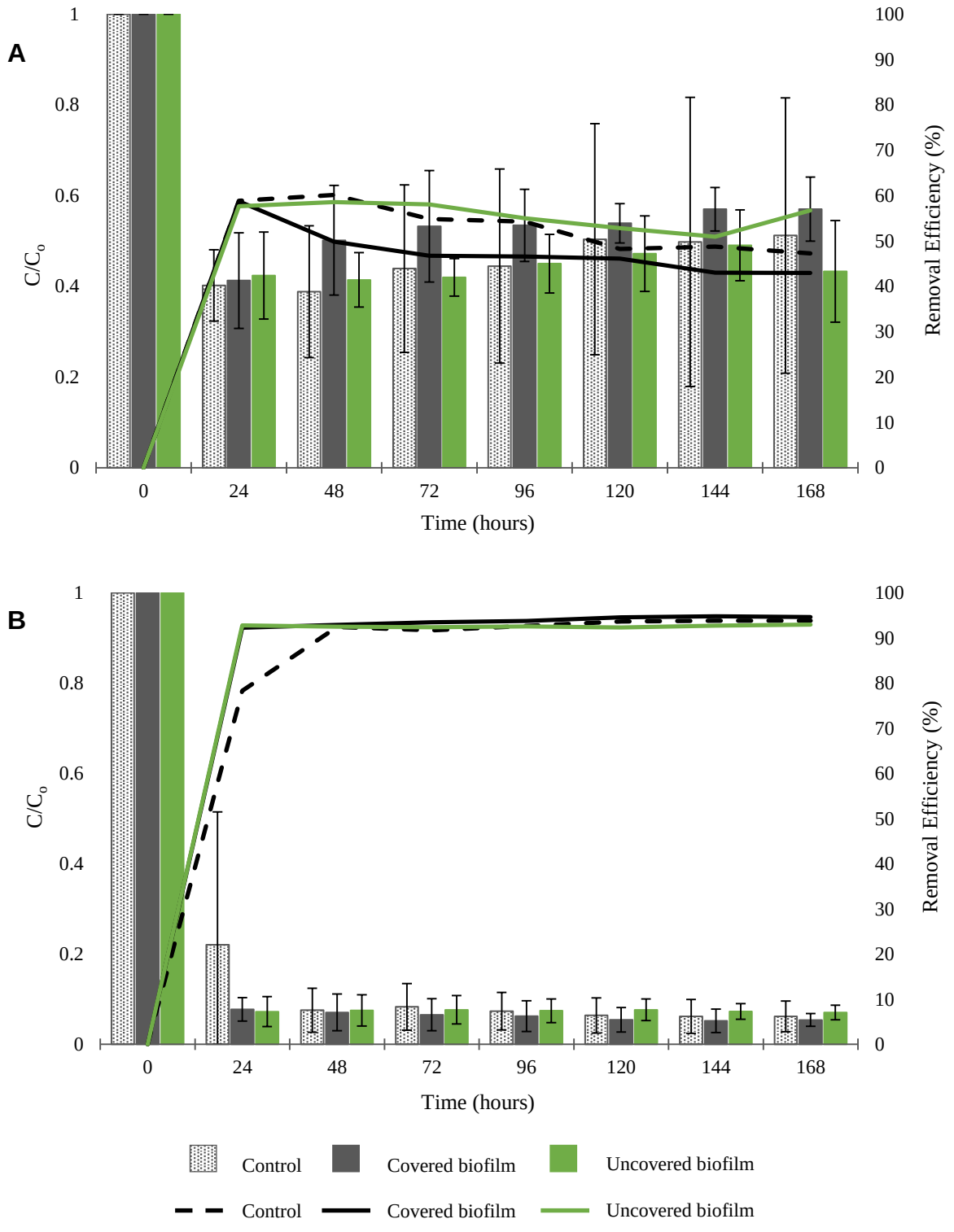

Figure 4-7. Normalised values of dissolved copper (C/C_o, bars) in influent (hour = 0) and treated effluent; also, the removal efficiency (%, lines) of dissolved copper shown on the second axis. (A) Recycled glass and (B) mussel shell substrates with three different treatments; treatment 1 = control (no biofilm), treatment 2 = covered biofilm (biofilm grown in dark conditions), and treatment 3 = uncovered biofilm (biofilm grown in light conditions).

4.4.2.3 Lead

Lead was successfully treated in all experiments (Table 4-9). Influent lead concentrations showed slight variation across all experimental runs, with concentrations ranging between 30.3 ± 2.2 µg/L to 35.2 ± 5.3 µg/L; the target concentration was 41.8 µg/L. Within 24 hours of the beginning of the experiments, the concentration of lead across both substrates and all treatments had reduced to less than a tenth of its initial concentration.

Table 4-9. Dissolved lead concentrations (µg/L; mean ± SD) in influent (hour = 0) and treated effluent for the recycled glass and mussel shell substrates with three treatment conditions. Treatment conditions are no biofilm (substrate only, control; n = 4), biofilm grown with no light exposure (covered biofilm; n = 4), and biofilm grown exposed to light (uncovered biofilm; n = 4).

Time (hours)	Recycled glass			Mussel shell		
	Control	Covered biofilm	Uncovered biofilm	Control	Covered biofilm	Uncovered biofilm
0	31.4 ± 1.7	35.2 ± 5.3	34.5 ± 1.3	30.3 ± 2.2	34.0 ± 2.8	34.4 ± 1.8
24	1.4 ± 0.5	1.6 ± 0.2	1.6 ± 0.7	1.2 ± 1.0	0.8 ± 0.3	0.8 ± 0.3
48	1.4 ± 0.5	1.5 ± 0.3	1.4 ± 0.5	0.5 ± 0.3	0.3 ± 0.1	0.5 ± 0.4
72	1.6 ± 0.6	1.8 ± 0.5	1.4 ± 0.6	0.6 ± 0.5	0.4 ± 0.3	0.4 ± 0.3
96	1.5 ± 0.7	1.7 ± 0.7	1.4 ± 0.6	0.5 ± 0.3	0.5 ± 0.5	0.3 ± 0.04
120	1.9 ± 0.9	2.1 ± 1.1	1.6 ± 0.7	1.2 ± 1.5	0.3 ± 0.2	0.3 ± 0.1
144	1.6 ± 0.8	2.4 ± 1.3	1.8 ± 0.7	0.4 ± 0.4	0.4 ± 0.4	0.3 ± 0.1
168	2.0 ± 1.2	2.8 ± 2.1	1.3 ± 0.8	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.2

All three recycled glass treatments reached virtually identical lead concentrations after just 24 hours. Concentrations of dissolved lead stayed consistent between treatments for the majority of the experiment. Towards the end of the experiment some variation in the treatments can be seen. For example, the covered biofilm treatment showed a very slight increase to 2.8 ± 2.1 µg/L from 1.7 ± 0.7 µg/L in lead concentration, and while the control saw a similar increase, the uncovered biofilm treatment continued to decrease in dissolved copper concentrations between 144 and 168 hours.

Dissolved lead concentrations dropped dramatically in the mussel shell experiments, and in all but two samples, the concentration of lead was below 1 µg/L, demonstrating a substantial reduction. Such low concentrations of lead were seen after just 24 hours and stayed at these concentrations for the remainder of the experiment. Furthermore, there was no discernible difference, in lead concentrations, between the control and either of the biofilm treatments over time.

Removal Efficiency

After an initial peak at 24 hours, all recycled glass treatments displayed a stable trend in removal efficiency over the course of the experiment (Figure 4-8 A), and all treatments showed excellent removal abilities with over 90% of the dissolved lead in these experiments being treated. The trend was most prominent in the control and covered biofilm treatments, which followed similar removal rates up until 120 hours. The uncovered biofilm treatment showed some variation but generally stayed stable, ranging between 95% to 96% removal efficiency for the duration of the experiment.

All mussel shell treatments showed excellent removal abilities with over 98% removal of dissolved lead being achieved (Figure 4-8 B). After just 24 hours both biofilm treatments showed identical removal efficiencies of 98%. Both biofilm treatments maintained very similar removal efficiencies for the remainder of the experiment and overall showed marginally better results than the control treatment. Despite a slightly lower removal efficiency at 24 hours, of 96%, the control treatment achieved over 98% removal efficiency for much of the experiment. Therefore, there is very little observable difference between all of the mussel shell treatments for removal of dissolved lead.

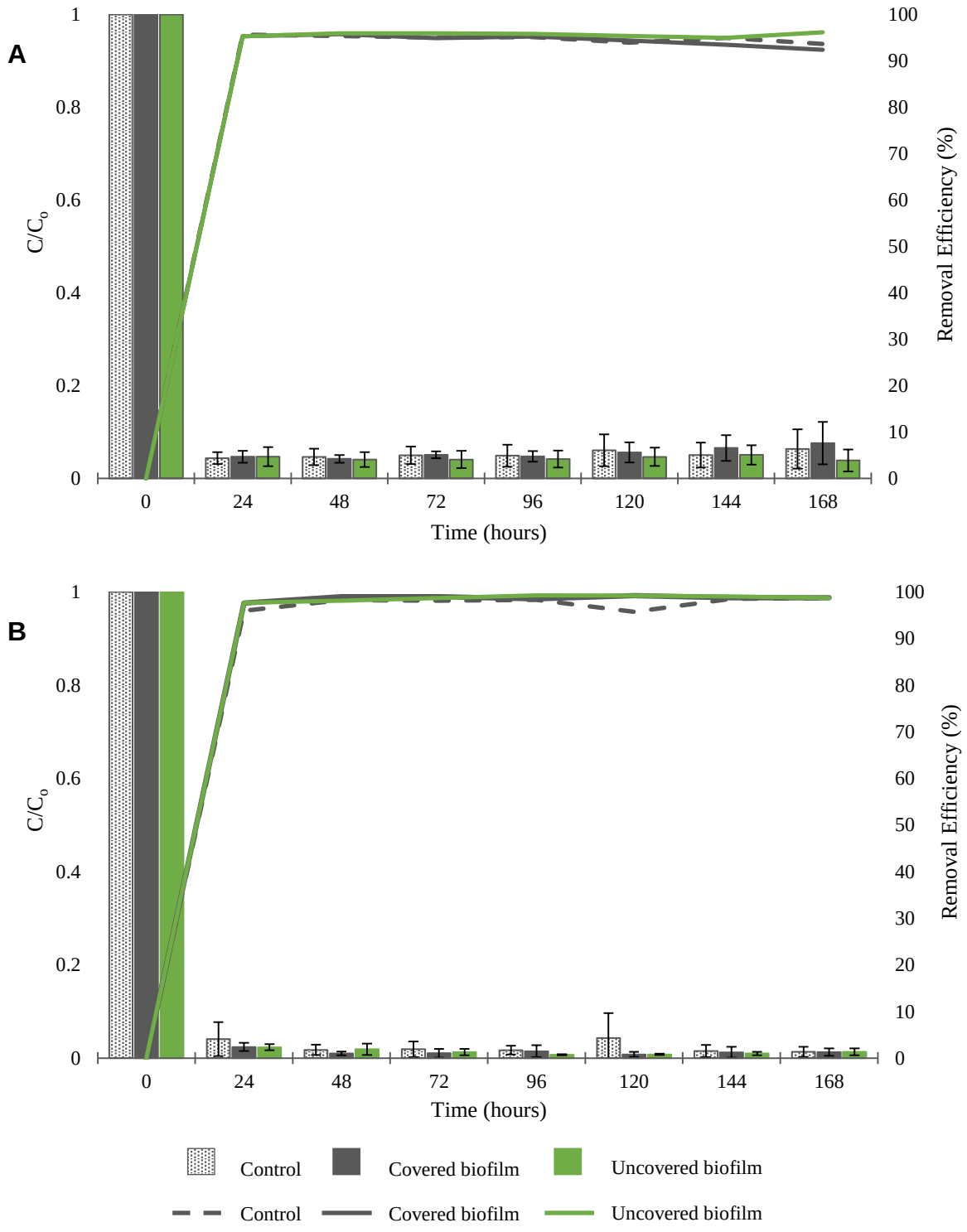

Figure 4-8. Normalised values of dissolved lead (C/C_o, bars) in influent (hour = 0) and treated effluent; also, removal efficiency (%, lines) of dissolved lead shown on the second axis. (A) Recycled glass and (B) mussel shell substrates with three different treatments; treatment 1 = control (no biofilm), treatment 2 = covered biofilm (biofilm grown in dark conditions), and treatment 3 = uncovered biofilm (biofilm grown in light conditions).

4.5 Phase Two – Whole Column Biofilm Experiments

4.5.1 Effluent Characterisation

Water quality parameters, pH, specific conductance, and alkalinity, were measured throughout the experiments. The pH and specific conductance were measured initially and then every 2, 4, 6, 12, 24 and 48 hours after the beginning of the experiment. Total alkalinity was measured at the beginning (influent; hour = 0) and end of the experiments (hour = 48). Influent characterisation is presented in section 4.5, Table 4-4 and Table 4-5 and a summary of the mean ± SD pH, specific conductance, and alkalinity is shown in Table 4-10 (see Appendix B for full set of water quality results). Substantial differences can be seen between substrates for all of the above characteristics. Please note, phase two experiments were conducted using a single type of biofilm that was grown in light conditions.

The pH of both the mussel shell and recycled glass effluent can be considered slightly alkaline (influent pH = 5.5 ± 0.5). Mussel shell biofilm treatment (n = 24 measurements), achieved a pH that was consistently above 9 (range = 9.1 – 9.8) after 2 hours and remained consistent for the duration of the experiment. The pH of the mussel shell control treatment (n = 7 measurements) was more neutral with an average pH of 7.3 (range = 6.6 – 7.7). In the recycled glass biofilm treatment (n = 24 measurements), the pH was slightly less than that of the mussel shell biofilm treatment, and showed some variation over time; however, it maintained an alkaline pH (range = 7.4 – 8.9). As with the mussel shell, the recycled glass control treatment (n = 7 measurements) had a lower pH than the biofilm treatment (range = 6.7 – 7.6).

Specific conductance differed considerably between the recycled glass and mussel shell substrates, with both substrates increasing in specific conductance compared to the influent (influent specific conductance = 11.8 ± 0.3 µS/cm). This increase is noteworthy for the mussel shell substrate, which increased fourfold within the first 2 hours, in both the control (n = 1 measurement) and biofilm (n = 3 measurements) treatments. This level of increase plateaued slightly between 4 and 12 hours before continuing to increase for the remainder of the experiment. The recycled glass substrate showed a modest increase in specific conductance, more so in the biofilm treatment (n = 3 measurements) than the control treatment (n = 1 measurement) over the duration of the experiment. After 48 hours the biofilm treatment reached a specific conductance of approximately 80 µS/cm compared to the control treatment which reached 43 µS/cm.

Total alkalinity concentrations were almost twice as high for the mussel shell treatments compared to the recycled glass. Given that the influent of both substrates had a total alkalinity of just 14.7 ± 10.9 mg/L as $CaCO_3$ both substrates produced substantial increases in total alkalinity. The recycled glass biofilm treatment (n = 3 measurements) obtained concentrations of 234.9 ± 7.6 mg/L as $CaCO_3$ after 48 hours compared to the control treatment (n = 1 measurement) which reached just 18.7 mg/L as $CaCO_3$.

The mussel shell biofilm treatment had similar alkalinity as the equivalent recycled glass biofilm treatment (n = 3 measurements), attaining an alkalinity of 252.1 ± 2.6 mg/L as $CaCO_3$ while the mussel shell control treatment (n = 1 measurement) reached just 115.0 mg/L as $CaCO_3$.

Table 4-10. Water quality parameters in treated effluent from treatments with biofilm (treatment) and without biofilm (control) experiments for both recycled glass and mussel shell.

Parameter	Time measured (hours)	Mean value ± SD			
		Recycled glass		Mussel shell	
		Without biofilm (control)	With biofilm (treatment)	Without biofilm (control)	With biofilm (treatment)
pH	2 – 48	7.2	8.1 ± 0.5	7.3	9.5 ± 0.3
Specific conductance (µS/cm)	2	42.5	57.0 ± 6.5	137.6	284.9 ± 7.6
	24	42.7	79.1 ± 7.9	392.7	394.7 ±2.7
Alkalinity (mg/L as $CaCO_3$)	48	18.7	234.9 ± 7.6	115.0	252.1 ± 2.6

4.5.2 Effluent Metal Concentrations

These experiments were conducted to better understand the removal of dissolved heavy metals within the first 24 hours, since the layered biofilm experiments identified the first 24 hours as being crucial to dissolved heavy metal removal. Both treatments showed dissolved heavy metal removal abilities, as demonstrated by decreasing concentrations of the three key heavy metals and high removal efficiency values.

4.5.2.1 Zinc

The results for both the recycled glass and mussel shell treatments clearly showed the removal of dissolved zinc within the first 24 hours (Table 4-11). Influent dissolved zinc concentrations showed some variation across all experiments, ranging from 272.2 µg/L to 303.7 µg/L (target concentration = 280.2 µg/L).

Table 4-11. Dissolved zinc concentrations (μg/L; mean ± SD) in influent (hour = 0) and treated effluent for the recycled glass and mussel shell. Treatment conditions are control (substrate only, no biofilm; n = 1) and whole column biofilm (grown in light conditions; n = 3).

Time (hours)	Recycled glass		Mussel shell	
	Control	Biofilm	Control	Biofilm
0	278.5	272.2 ± 8.7	303.7	287.6 ± 11.90
2	188.0	160.6 ± 11.6	208.0	155.4 ± 4.9
4	169.2	76.3 ± 5.9	151.6	56.9 ± 3.5
6	180.7	39.85 ± 2.9	72.6	27.1 ± 0.7
12	156.9	31.0 ± 8.6	8.8	13.0 ± 0.7
24	133.1	47.6 ± 5.9	2.6	5.4 ± 1.3
48	49.2	35.9 ± 11.5	3.2	9.3 ± 2.8

A comparison between the recycled glass treatments showed that the biofilm treatment consistently achieved lower concentrations of dissolved zinc than the control treatment. Although, after just 2 hours both treatments demonstrated a reduction in dissolved zinc concentrations. After an initial decrease in the dissolved zinc concentration of approximately 90 μg/L the control treatment showed only a minor reduction in zinc between 4 and 24 hours, and then another substantial reduction between 24 and 48 hours of approximately 80 μg/L. The biofilm treatment, on the other hand, showed a steady decrease in dissolved zinc concentrations between 2 and 12 hours (160.6 ± 11.6 μg/L to 31.0 ± 8.6 μg/L), after which there was a 15 μg/L increase at 24 hours followed by a further small decrease at 48 hours.

Both mussel shell treatments showed significant decreases in dissolved zinc concentrations over 48 hours. Again, both treatments showed significant reductions within the first 2 hours, with the biofilm treatment decreasing by approximately half of its initial concentration. This considerable decrease continues from 2 to 12 hours, at which time the concentration of dissolved zinc stayed stable for the remainder of the experiment, ranging between 13.0 ± 0.7 μg/L and 5.4 ± 1.3 μg/L. The control mussel shell treatment showed a very similar trend to the biofilm treatment, such that between 2 and 12 hours the concentration of dissolved zinc decreased significantly from an initial concentration of 303.7 μg/L to 8.8 μg/L. Between 12 and 48 hours, the concentrations did not substantially change any further but reach a minimum of 2.6 μg/L at 24 hours after the start of the experiment, thus displaying a substantial reduction in dissolved zinc concentrations.

Removal Efficiency

Both substrates displayed excellent dissolved zinc removal efficiencies across both treatments, with peak removal achieved within 6 to 12 hours of the beginning of the experiment.

The recycled glass with biofilm treatment showed considerably greater removal efficiency than the control treatment (Figure 4-9 A). Within 6 hours the biofilm treatment reached and maintained removal efficiencies of over 80%, with the greatest removal efficiency of 88% recorded at 12 hours. The control treatment displayed a much different trend. After just 2 hours both the recycled glass treatments showed similar removal efficiencies, however, between 4 and 24 hours the control treatment removal efficiency only increased by approximately 23%, unlike the biofilm treatment which showed an increase of 48% for the same period. Between 24 and 48 hours the control treatment removal efficiency increased significantly such that both treatments (control and biofilm) finished the experiment with similar percent removal (control = 82% and biofilm = 87%).

The difference between mussel shell treatments was less substantial than that seen in the recycled glass experiments, yet, overall the mussel shell achieved a higher removal efficiency over time (Figure 4-9 B). The biggest difference in treatments was seen after 4 hours (control = 50% and biofilm = 80%). To begin with, the biofilm treatment showed the best removal efficiency, reaching 91% removal after 6 hours. However, at 12 hours after the start of the experiment both mussel shell treatments showed very similar removal efficiencies, with the control treatment reaching 97% and the biofilm treatment reaching 98%. The similarities in removal efficiencies continued for the remainder of the experiment.

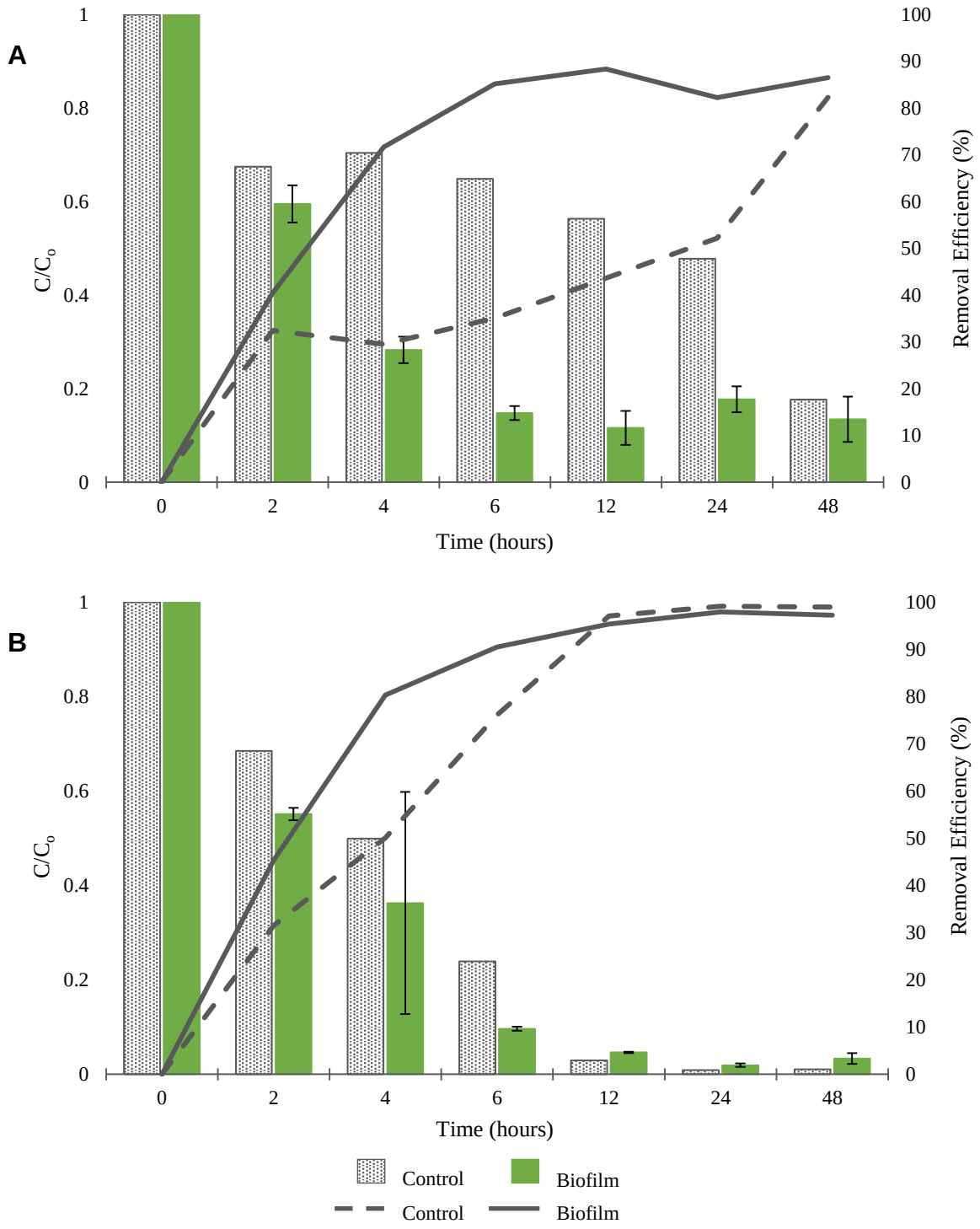

Figure 4-9. Normalised values of dissolved zinc (C/C_o, bars) in influent (hour = 0) and in treated effluent thereafter; also, removal efficiency (%, lines) of dissolved zinc shown on the second axes. (A) Recycled glass and (B) mussel shell substrates with two different treatments; treatment 1 = control (no biofilm), and treatment 2 = whole column biofilm (grown in light conditions).

4.5.2.2 Copper

Dissolved copper concentrations decreased significantly across both substrates and treatments (Table 4-12). Influent concentrations showed very little variation with a difference of just 2.9 µg/L between the maximum and minimum concentration (target concentration = 154.0 µg/L).

Table 4-12. Dissolved copper concentrations (µg/L; mean ± SD) in influent (hour = 0) and treated effluent for the recycled glass and mussel shell substrates. Treatment conditions are control (substrate only, no biofilm; n = 1) and whole column biofilm (grown in light conditions; n = 3).

Time (hours)	Recycled glass		Mussel shell	
	Control	Biofilm	Control	Biofilm
0	111.8	110.4 ± 2.7	111.1	108.9 ± 2.2
2	55.3	69.7 ± 4.3	79.7	73.6 ± 3.3
4	31.8	43.6 ± 3.7	61.9	43.5 ± 4.4
6	25.4	32.4 ± 3.3	32.2	27.2 ± 5.5
12	22.3	31.3 ± 5.3	6.7	22.3 ± 5.4
24	19.3	31.9 ± 6.9	3.2	20.5 ± 5.5
48	19.8	35.2 ± 3.4	3.4	18.9 ± 5.6

Dissolved copper concentrations did not decrease to the same levels in the recycled glass control and biofilm treatments, although they followed a very similar trend. Within the recycled glass substrate, the biggest decrease in dissolved copper occurred in the control treatment, with concentrations halved in the first 2 hours, from 111.8 µg/L to 55.3 µg/L. After this a steady decrease occurred which at 24 hours stabilised at 19.0 µg/L until the end of the experiment. The biofilm treatment did not remove dissolved copper from the synthetic stormwater to the same level as the control treatment, showing consistently higher concentrations. Within the biofilm treatment a decrease in the first 4 hours, from 110.4 ± 2.7 to 43.6 ± 3.7 µg/L, was followed by a comparatively stable period which persisted for the remainder of the experiment. Such that between 6 and 48 hours, the concentration of dissolved copper fluctuated between 31.3 ± 5.3 µg/L and 35.2 ± 3.4 µg/L, showing no further reductions in dissolved zinc concentrations.

Both mussel shell treatments showed a good decreasing trend in dissolved copper concentrations, however, over time variation between treatments became more obvious. At the beginning of the experiment (2 and 6 hours) concentrations between the two treatments were fairly similar, with a difference of only 5 µg/L between the control and biofilm treatment at 6 hours. However, from hour 6 until the end of the experiment concentrations of dissolved copper began to differ, with the control treatment demonstrating a much greater decrease in concentrations compared to the biofilm treatment.

Removal Efficiency

Both substrates demonstrate a removal ability with the mussel shell treatments consistently reaching over 80% removal.

Positive removal trends could be seen in both recycled glass treatments (Figure 4-10 A). An exception to this was in the biofilm treatment where after 24 hours the removal efficiency began to decrease, from a maximum at 12 hours of 71% to 68% after 48 hours. The control treatment consistently obtained higher removal rates for dissolved copper than the biofilm treatment, with a maximum of 83% at 24 hours.

Both mussel shell treatments showed positive removal efficiencies for the duration of the experiment (Figure 4-10 B). Interestingly, the removal efficiency of both treatments remained fairly similar for the first six hours (control = 71% and biofilm = 75%). Thereafter the treatments displayed marginally different trends and the control treatment surpassed the biofilm as the most efficient treatment and continued to increase until it levelled out at 24 hours with a maximum removal efficiency of 97%. The biofilm treatment also continued to increase, however, to a lesser degree and reached a maximum removal of dissolved copper after 48 hours (83%).

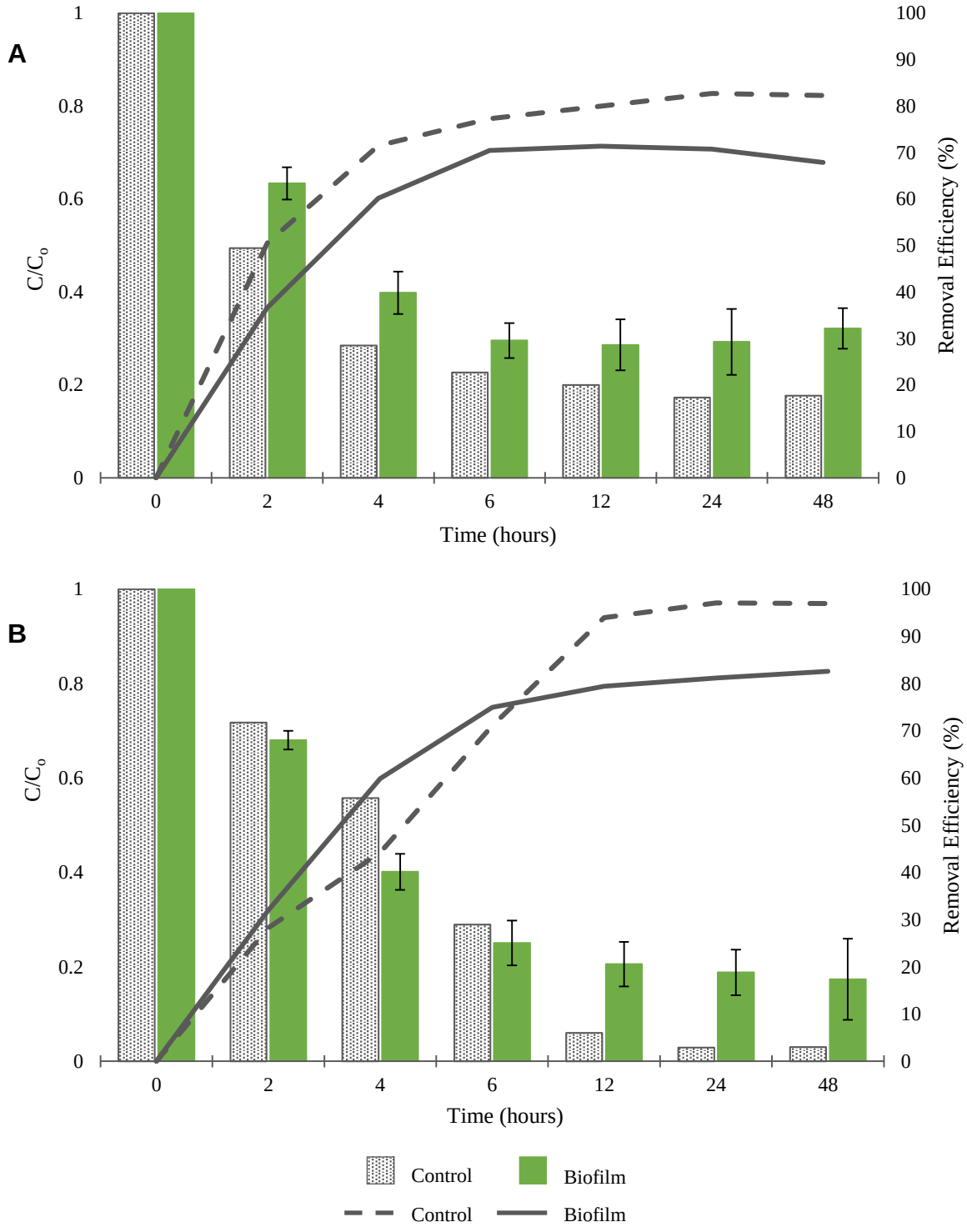

Figure 4-10. Normalised values of dissolved copper (C/C_o, bars) in influent (hour = 0) and in treated effluent thereafter; also, removal efficiency (%, lines) of dissolved copper shown on the second axis. (A) Recycled glass and (B) mussel shell substrates with two different treatments; treatment 1 = control (no biofilm), and treatment 2 = whole column biofilm (grown in light conditions).

4.5.2.3 Lead

Dissolved lead was effectively treated by both substrates and achieved concentrations below 3 µg/L in all experiments, after 48 hours (Table 4-13). To begin with, influent concentrations were very similar, with a difference of just 1.8 µg/L of dissolved lead between the maximum and minimum concentrations (target concentration = 41.8 µg/L).

Table 4-13. Dissolved lead concentrations (µg/L; mean ± SD) in influent (hour = 0) and treated effluent for the recycled glass and mussel shell substrates. Treatment conditions are control (substrate only, no biofilm; n = 1) and whole column biofilm (grown in light conditions; n = 3).

Time (hours)	Recycled glass		Mussel shell	
	Control	Biofilm	Control	Biofilm
0	31.7	31.1 ±1.1	30.8	29.9 ± 0.0
2	12.7	17.3 ± 2.0	21.3	16.1 ± 0.9
4	6.2	9.9 ± 1.1	15.5	6.7 ± 1.0
6	3.3	6.0 ± 1.0	8.4	2.8 ± 0.1
12	2.1	4.4 ± 1.2	1.7	1.5 ± 0.0
24	0.9	3.7 ± 1.4	0.3	0.4 ± 0.1
48	0.7	2.6 ± 0.8	0.3	0.6 ± 1.2

Both recycled glass treatments reached very similar dissolved lead concentrations after just 2 hours and showed good positive trends for the duration of the experiment. However, overall, the control treatment consistently achieved the lowest concentrations when compared to the biofilm treatment, with the biggest difference, of 4.6 µg/L, seen after just 2 hours. After this the difference reduced slightly but the control treatment continued to have lowest concentrations of dissolved lead, reaching just 0.7 µg/L after 48 hours. Despite these variations, the biofilm treatment performed as well, with over a 10 fold reduction in dissolved lead concentrations after 48 hours.

Lead concentrations dropped dramatically in the mussel shell treatments. For much of the experiment, and in contrast to the recycled glass experiments, the biofilm treatment performed the best. This was seen up until 24 hours, when the biofilm and control treatments reached almost identical concentrations (biofilm = 0.4 ± 0.1 µg/L and control = 0.3 µg/L), after which there was no discernible difference in dissolved lead concentrations between the control or biofilm treatments.

Removal Efficiency

The removal efficiency of both substrates reached over 90% after just 12 hours (with one exception being the biofilm treatment in the recycled glass) demonstrating that both substrates can efficiently remove dissolved lead from a synthetic stormwater over time. Furthermore, in all cases, a positive trend was found in removal efficiency.

The addition of biofilm in the recycled glass columns did not prove to be beneficial to the overall removal of dissolved lead (Figure 4-11 A). At all sampling times, the control treatment showed a higher removal efficiency than the biofilm treatment. Despite not achieving the same level of removal as the control treatment, columns with biofilm successfully treated the synthetic stormwater for dissolved lead. Following almost identical trends, a maximum removal efficiency of 91% was reached after 48 hours in the biofilm treatment. These results suggest that the presence of the biofilm on the surface of the recycled glass may have hindered the removal of dissolved lead from the synthetic stormwater.

In all mussel shell treatments, over 94% removal of dissolved lead was achieved within the first 12 hours showing excellent removal abilities by both the mussel shell treatments (Figure 4-11 B). In comparison to the recycled glass experiments, initial removal efficiency is greatest in the biofilm treatments, however, after 12 hours the removal efficiency of both mussel shell treatments are effectively identical (control = 94% and biofilm = 95%) and remained this close for the duration of the experiment.

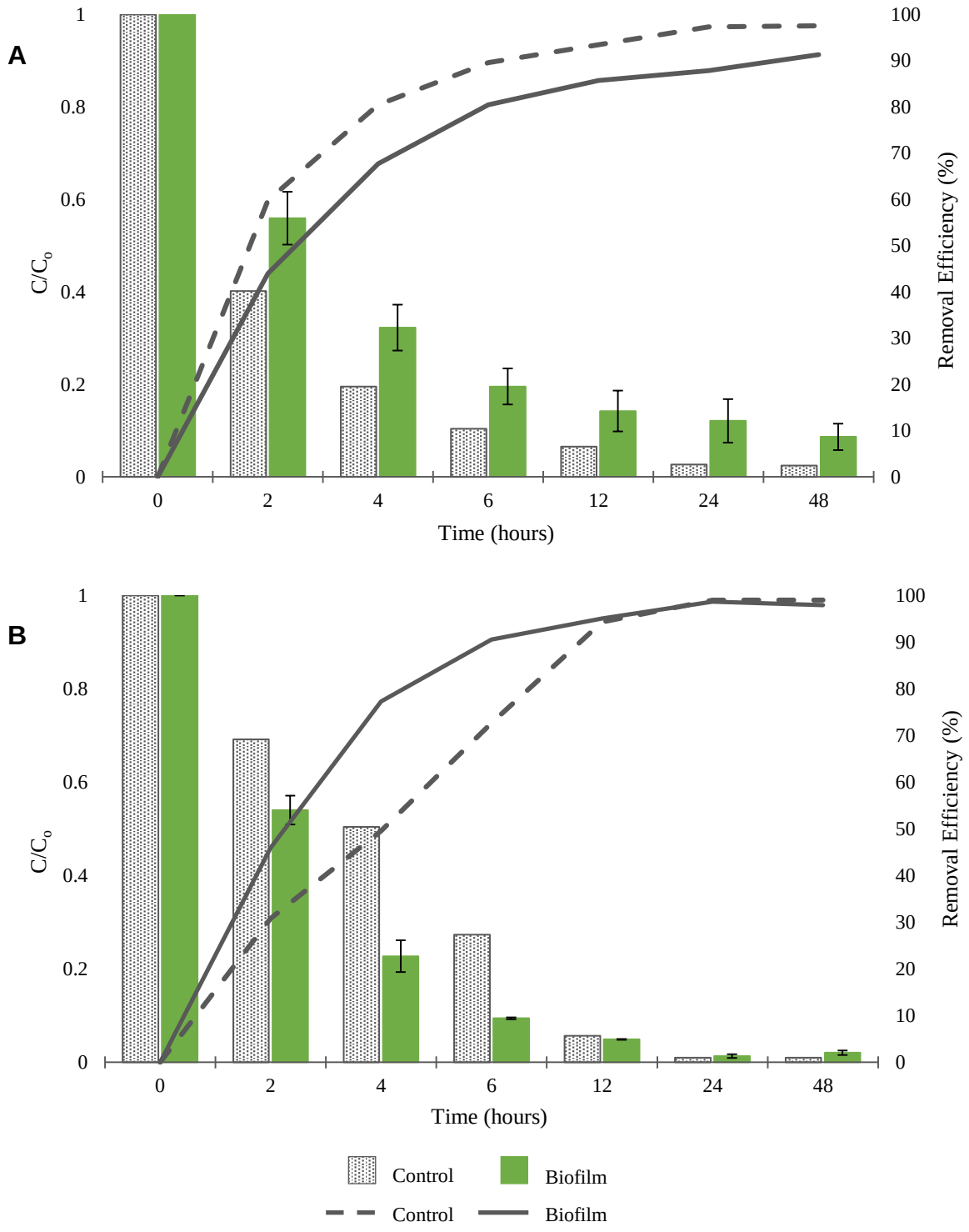

Figure 4-11. Normalised values of dissolved lead (C/C_o, bars) in influent (hour = 0) and in treated effluent thereafter; also, removal efficiency (%, lines) of dissolved lead shown on the second axis. (A) Recycled glass and (B) mussel shell substrates with two different treatments; treatment 1 = control (no biofilm), and treatment 2 = whole column biofilm (grown in light conditions).

4.5.3 Biofilm Digestion

Heavy metals accumulated within the biofilm to very high concentrations (Table 4-14), demonstrating the biofilm's ability to sorb heavy metals from aqueous environments.

Table 4-14. Results of biofilm digestion. Concentrations of zinc, copper, and lead within the biofilm, in the synthetic stormwater solution and their respective enrichment factor (comparison between the concentration of metal in the biofilm and in the water).

	Zinc	**Copper**	**Lead**
Biofilm (mg/kg dry weight)	620.7	362.1	65.5
Synthetic stormwater (mg/L)	0.28	0.11	0.03
Enrichment factor	2216	3292	2183

4.6 Gravel Experiments

Gravel experiments were conducted to provide background removal rates on an inert substrate, however the gravel also showed excellent dissolved heavy metal removal rates (Figure 4-12). Initial concentrations differed somewhat from target concentrations, where copper had an initial concentration of 112 µg/L with a target of 154 µg/L, zinc had an initial concentration of 275 µg/L with a target of 280 µg/L and lead had an initial concentration of 35 µg/L with a target of 42 µg/L. After just 4 hours concentrations of dissolved heavy metals had decreased significantly, with similarly high removal efficiencies attained. Zinc and lead both reached 99% removal after 12 hours, while copper reached 96% removal. These levels of removal were maintained for the majority of the experiment; however, copper did show a slight decreasing trend in removal efficiency as the experiment progressed. Despite this, gravel demonstrated an excellent removal efficiency that could be seen for all three heavy metals over time.

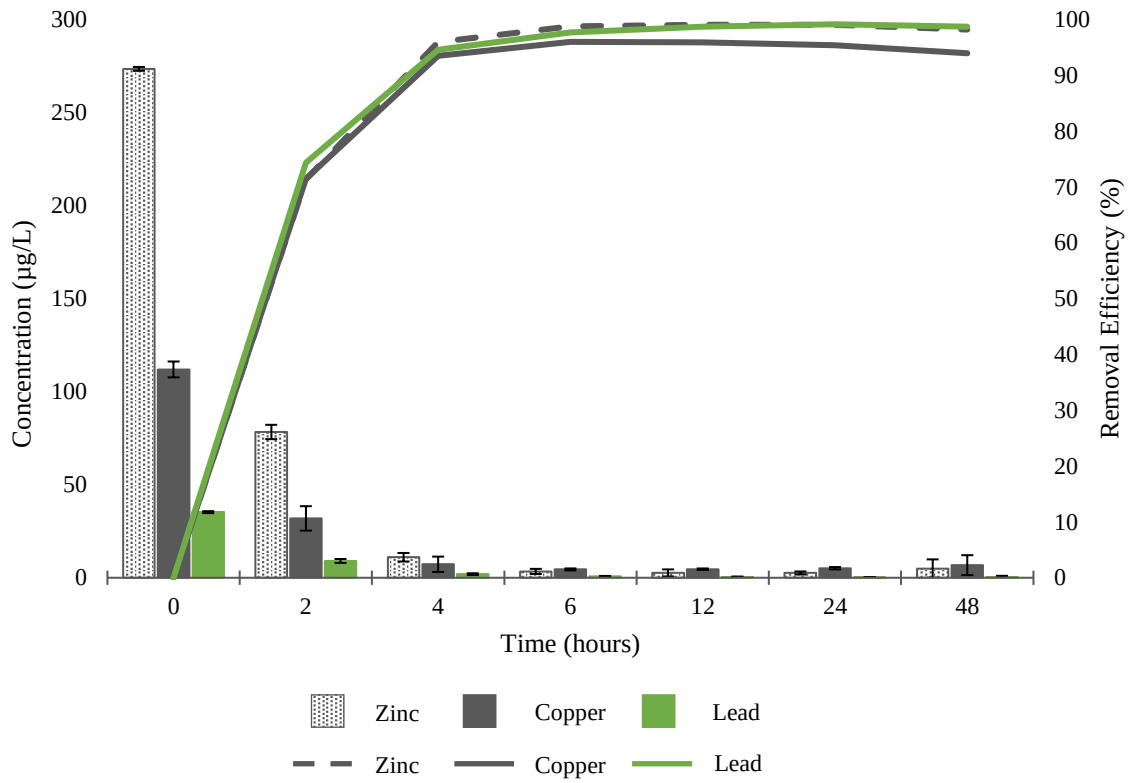

Figure 4-12. Results from the column experiments using gravel. Dissolved heavy metal concentrations (µg/L, bars) in influent (hour = 0) and in treated effluent thereafter; the second axis shows the removal efficiency (%, lines) of dissolved zinc, copper, and lead.

Water quality parameters, pH and specific conductance were measured throughout the gravel experiment, while alkalinity was measured at the beginning and end of the experiment. The effluent pH was neutral, with an average of 6.6 ± 0.2 over the 48 hours of the experiment, this had increased from a slightly acidic influent pH of 4.7 ± 0.1. Specific conductance did not increase significantly during the experiment. Influent specific conductance was 18.9 ± 0.3 µS/cm and rose slightly to a median value of 27.1 µS/cm over the duration of the experiment. Influent synthetic stormwater had no measurable alkalinity which increased slightly to 16.6 ± 3.6 mg/L as $CaCO_3$ after 48 hours exposure to the gravel substrate.

4.7 Batch Adsorption Experiments

Adsorption experiments were conducted to confirm the removal efficiencies of the recycled glass, mussel shell, and gravel. Overall, the results reflected the removal efficiencies achieved in the column experiments (Figure 4-13), confirming that all three substrates were capable of very good dissolved heavy metal removal. Gravel continued to show excellent removal efficiencies for all three heavy metals, in particular copper which achieved over 95% removal after 6 hours compared to just 78% and 56% in recycled glass and mussel shell respectively. Gravel also showed excellent results for zinc and lead achieving over 99% removal efficiencies after 48 hours.

Mussel shell achieved similar removal efficiencies to the gravel with regards to zinc (98%) and lead (99%) after 48 hours, however, copper was not removed to the same degree achieving just 83% during the same period. Lead removal reached very high levels after just 2 hours exposure to the mussel shell and maintained this efficiency for the remainder of the experiment. On the other hand, zinc took approximately 6 hours to reach a similar level of removal but maintained it thereafter. Despite the removal efficiency of copper increasing for the duration of the experiment it did not achieve the same level of removal as zinc and lead.

Recycled glass performed well for copper (86%) and zinc (92%) removal, with removal efficiencies very similar to that of the gravel and mussel shell. Lead on the other hand achieved a similar removal efficiency (98%) to gravel and mussel shell after 48 hours, however, it took only 12 hours exposure to the recycled glass to achieve this level of removal.

The only water quality parameter measured during the benchtop experiments was pH. Within the gravel experiments pH continued to increase for the duration of the experiments reaching a maximum pH of 8.2 ± 0.1 after 24 hours. Mussel shell displayed an interesting trend with the pH decreasing as the experiment progressed, such that after 2 hours exposure the pH was 8.4 ± 0.1 but by the end of the experiment (48 hours) it had decreased to 7.7 ± 0.1. Recycled glass did not display an obvious trend, instead it reached a pH of 8.4 ± 0.0 after 2 hours and stayed approximately the same (maximum pH = 8.7 ± 0.0 and minimum pH = 8.1 ± 0.2) for the duration of the experiment.

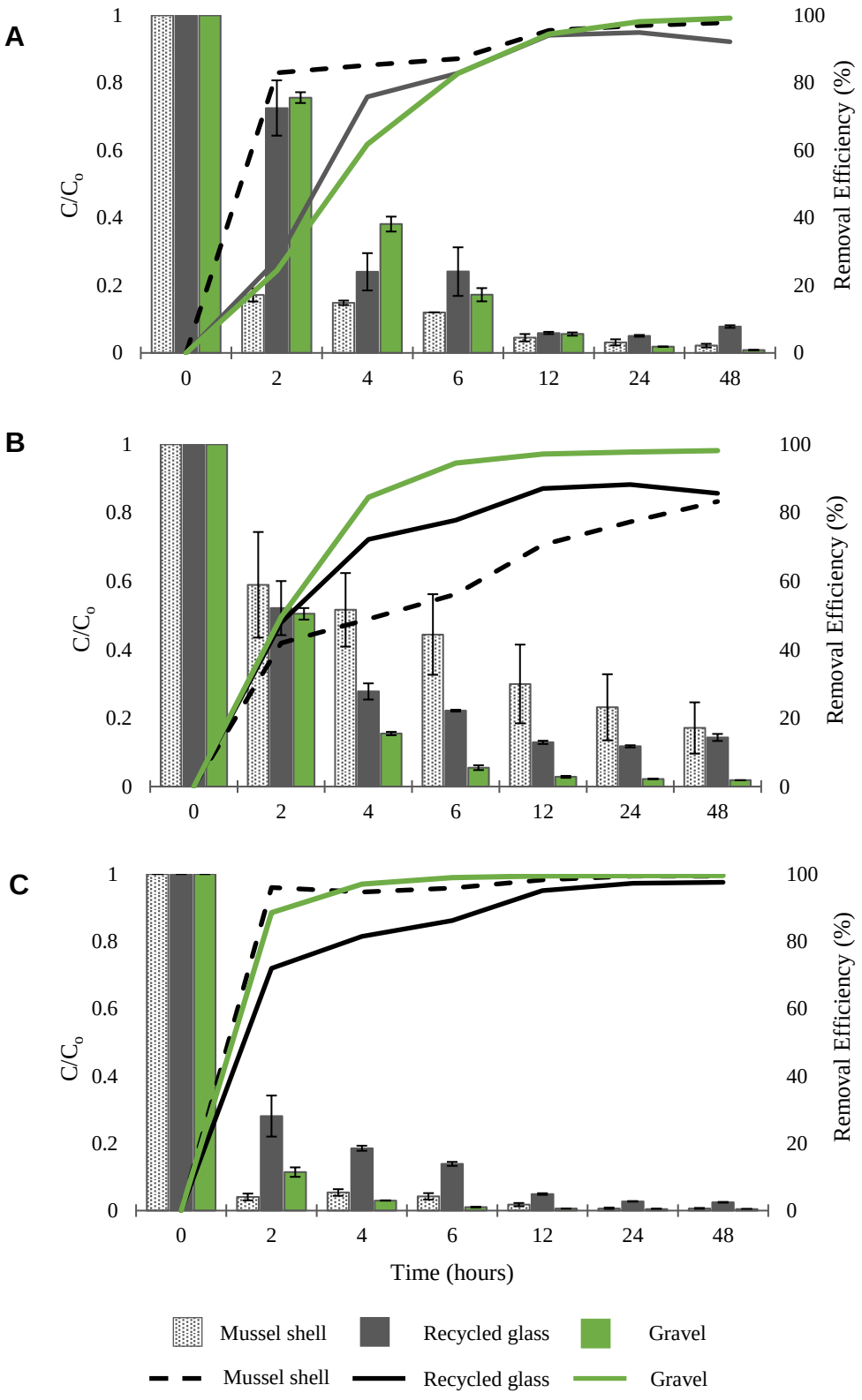

Figure 4-13. Results from the batch adsorption experiments. Normalised values (C/C_o, bars) for dissolved (A) zinc, (B) copper, and (C) lead concentrations in influent (hour = 0) and in treated effluent thereafter for the three substrates (n = 2 for each substrate); also shown on the second axes are the removal efficiencies (%, lines) of the substrates.

4.8 Affect of pH

pH is known to influence the partitioning of heavy metals in aqueous solutions, and the relationship between pH and dissolved heavy metal fractions is well known. Such that, by increasing the pH it may be possible to increase particulate metal speciation and enhance removal. In these experiments pH was measured for all experiments, however, for consistency the regression analyses below only show the results of the adsorption experiments (Table 4-15, Figure 4-14). By creating regression relationships between the removal efficiencies (of each substrate for each of the heavy metal contaminants) and pH, we can understand the affects of pH on the removal efficiencies of each substrate.

The relationship between the recycled glass and each of the three heavy metals was only weak and were not significant, as shown by the low R^2 values and high p values ($p < 0.05$). The relationship between mussel shell and the removal efficiency of each of the three heavy metals was strong and significant, in particular, zinc which had the highest R^2 value at 0.8336 and one of the lowest p values ($p = 0.0001$). Gravel showed a moderate relationship between substrate and heavy metal removal efficiency, with copper and lead having the highest R^2 values and in both cases the result was significant ($p < 0.05$). Zinc on the other hand had a low R^2 value and was not significant.

Table 4-15. Regression statistics for recycled glass, mussel shell, and gravel.

		Regression statistics			
		Observations (n)	*df*	R^2	Significance (P)
Recycled glass	Zinc	10	1	0.0815	0.4238
	Copper	10	1	0.2921	0.1067
	Lead	12	1	0.2511	0.2595
Mussel shell	Zinc	11	1	0.8336	0.0001*
	Copper	12	1	0.7878	0.0001*
	Lead	12	1	0.7313	0.0004*
Gravel	Zinc	11	1	0.3500	0.0552
	Copper	12	1	0.5534	0.0055*
	Lead	12	1	0.7413	0.0058*

Note: * indicates significance to an alpha level of 5%

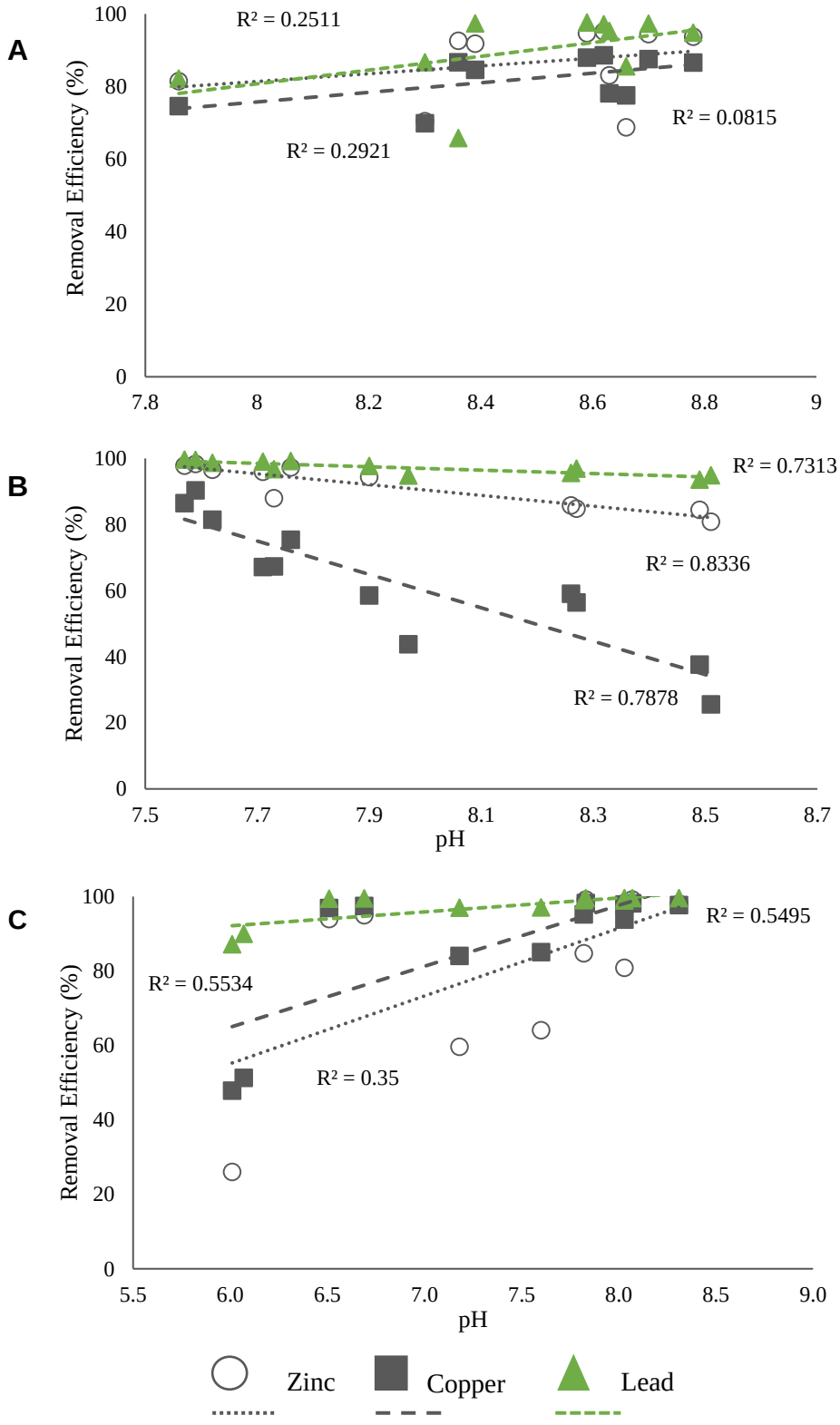

Figure 4-14. Regression trends for the removal efficiencies of (A) recycled glass, (B) mussel shell, and (C) gravel substrates as a function of synthetic stormwater effluent pH (benchtop experiments only). Note the X axes do not start at 0.

Chapter 5. Discussion

The results presented in chapter 4 are discussed in this chapter with an emphasis on the role of biofilm in the sorption of dissolved heavy metals from stormwater. Furthermore, the influence of gravel, recycled glass, and mussel shell on the removal of dissolved heavy metals, and the mechanisms of removal are discussed.

5.1 Sorption of Dissolved Heavy Metals by Biofilm

Microscope images of the biofilm grown in light conditions (phase two experiments) show a dense freshwater autotrophic biofilm with heterogeneous microcolonies. Filamentous blue-green algae (Cyanobacteria), green algae (Chlorophyta), diatoms (Bacillariophyceae), and bacteria dominated the composition of the biofilm. Initially, the aim of this thesis was to make comparisons between the heavy metal removal abilities of biofilm grown in light and dark conditions, however, slow biofilm growth in dark conditions meant that only the biofilm grown in light conditions was used.

Light conditions result in a biofilm which is greater in biomass, thickness, and species richness when compared to biofilm grown in dark conditions (Sekar et al., 2002). Furthermore, Sekar et al. (2002) identified a three-phase pattern of succession in light-grown biofilm, with initial colonisation by Chlorophyta followed by Bacillariophyceae, and finally Cyanobacteria. This colonisation pattern supports the dominant composition of the light grown biofilm seen in this study. Biofilm grown in dark conditions, on the other hand, are dominated by diatoms and bacteria, particularly Cyanobacteria (Sekar et al., 2002).

Natural biofilm collected from a filter drain showed a similar composition to biofilm grown in dark conditions, in that it was dominated by bacteria, in particular, Cyanobacteria (71%) (Feder et al., 2015). Therefore, the biofilm grown in the current study differs in composition to what may be expected to grow in stormwater treatment systems, because it contains the autotrophic microorganisms Chlorophyta and Bacillariophyceae. However, it is likely that biofilm grew on the inner substrate surfaces of the columns (not exposed to light) which would have better replicated the dark environment of stormwater treatment systems. Additionally, this study was limited in that the identification of bacteria phylum was not possible, although, it is highly probable that bacteria were present in the biofilm grown in this study (e.g. Feder et al., 2015).

The freshwater biofilm grown in this study (during phase two experiments) demonstrated an ability to sorb dissolved heavy metals from an aqueous solution with typical stormwater concentrations of dissolved zinc, copper, and lead. Adsorbed zinc reached the highest concentration within the biofilm with 620 mg/kg of dry biofilm weight, followed by copper with 360 mg/kg of dry biofilm weight, and

lastly lead with 70 mg/kg of dry biofilm weight. These concentrations were achieved after 48 hours exposure to the synthetic stormwater. Unfortunately, the initial concentration of heavy metals within the biofilm was not measured, but initial biofilm growth occurred without known sources of heavy metal contributions and initial concentrations of heavy metals are therefore assumed to be negligible.

The ability of biofilm to sorb heavy metals from aqueous solutions has been demonstrated in a range of environments from wastewater and stormwater (e.g. Costley et al., 2001; Feder et al., 2015) to freshwater (e.g. Farag et al., 2007; Ancion et al., 2010; Ancion et al., 2013). Biofilms employ a variety of mechanisms to sorb heavy metal from aqueous solutions. These include active processes such as bioaccumulation, biomineralization, and biotransformation; and passive processes such as biosorption which incorporates physicochemical removal mechanisms (van Hullebusch et al., 2003; Feder et al., 2015). A similar study by Ancion (2010) reported concentrations of zinc, copper, and lead in a freshwater biofilm to be 204.3, 74.6, and 153.4 mg/kg wet weight respectively after 21 days exposure to a highly-contaminated synthetic urban runoff. Enrichment factors given by the same study showed the biofilm grown accumulated very high concentrations of heavy metals, up to 500:1, 1500:1, and 6000:1 for zinc, copper, and lead respectively (Ancion, 2010). Those authors attributed these concentrations to a long exposure time (21 days) with an equilibrium (defined as the concentration of metals in the water and biofilm in a steady state) not reached before 7 to 14 days, suggesting a more permanent metal accumulation process than biosorption alone (Ancion, 2010). The biofilm in the present study had enrichment factors much greater than those reported by Ancion (2010), with the exception of lead, despite being exposed for a significantly shorter period of time. This may be attributed to the differing removal processes occurring on different time scales. For example, biosorption is a rapid process which can reach equilibrium within an hour of exposure, compared to more permanent accumulation processes which can take much longer (Hu et al., 2003; Hu et al., 2005; Ancion, 2010). Additionally, the rate of biosorption can decrease with increasing heavy metal concentrations due to competition between ions for binding sites on the biofilm surface (Jang et al., 2001; Feder et al., 2015).

Within this study, the enrichment factor results suggested a preferential binding of copper ions by the biofilm, with the affinity of the heavy metal ions in the order of Cu>Zn>Pb, which differs from other studies. Ancion (2010) found that biofilm had an affinity for lead over both copper and zinc, and Feder (2014) found that biofilm-covered gravel removed heavy metals in the order of Pb>Cu>Zn compared to clean gravel (no biofilm) which showed an affinity in the order of Zn>Pb>Cu. The differences seen between this study and earlier studies may in part be due to the composition of the biofilm, the growth substrate, and the concentration of heavy metals to which the biofilm is exposed.

5.1.1 Biofilm Coated Substrate

Despite biofilm demonstrating an ability to sorb dissolved heavy metals, when columns containing biofilm were compared to those which did not contain biofilm (phase two – whole column biofilm experiments), the difference in removal efficiency was minimal to non-existent, regardless of the amount of biofilm present. Therefore, biofilm did not have a significant impact on heavy metal removal relative to the substrate on its own.

Mussel shell consistently reached over 90% removal efficiency for all three heavy metals regardless of whether biofilm was present or not. When biofilm was present the mussel shell columns had an order of efficiency of Pb (98%) > Zn (97%) > Cu (83%), and without the biofilm, the order of efficiency was the same Pb (98%) ≥ Zn (98%) > Cu (97%). However, columns without biofilm showed a much higher removal efficiency of copper, achieving over 10% greater removal when compared to the columns containing biofilm.

The recycled glass showed good removal abilities, however, had greater variation within and between treatments. When biofilm was present it demonstrated an order of efficiency as follows Pb (91%) > Zn (87%) > Cu (68%). Without biofilm, the removal efficiency had an order of efficiency of Pb (98%) > Zn (82%) ≥ Cu (82%). In the columns without biofilm the removal efficiency of zinc decreased by approximately 5% compared to the columns containing biofilm. Additionally, the percentage removal of Cu increased by 15% when no biofilm was present.

Though not substantial, differences in removal efficiencies between control and biofilm treatments can be seen. For example, with mussel shell the removal efficiencies of lead and zinc do not change between treatments; however, the removal efficiency of copper significantly increased, by 14%, in the control treatments when compared to the biofilm treatments, suggesting that the biofilm inhibits the removal of copper by the mussel shell. A similar trend can be seen in the recycled glass experiments. Control treatments of recycled glass showed enhanced removal of lead (+ 7%) and copper (+ 14%) when compared to the biofilm treatment; however, zinc removal increased slightly by 5% in the presence of biofilm when compared to the control treatment, which was not seen in the mussel shell experiments. Despite this, it appears that the presence of biofilm actually inhibits the removal of dissolved heavy metals in both the recycled glass and mussel shell substrates. However, in some cases, as illustrated above, the removal of heavy metal ions may depend on the type of metal and the substrate the biofilm is grown on.

This phenomenon, where the biofilm effectively occupies and smoothers the reactive sites of the substrate leading to decreased adsorption, has been demonstrated by Kulczycki et al. (2005) and Anderson et al. (2006). In particular, Anderson et al. (2006) reported the adsorption capacity of granite rock with no biofilm to be up to 88% greater than rock covered with biofilm.

However, a study by Feder et al. (2015) reported the opposite, whereby they found that biofilm grown on a gravel substrate immobilised up to 29% more heavy metals than experiments with no biofilm. Furthermore, they found notable increases in the removal of lead (+14%) and zinc (up to 8%) when biofilms were present, which they presented as evidence that microorganisms can sequester metals more readily than the gravel surface alone (Feder et al., 2015). Thus, the increase in zinc removal seen in this study may be evidence that the biofilms were removing heavy metals from the synthetic stormwater, however, not as much as the substrate itself. Feder et al. (2015) attributed this increased removal to the active and passive cellular processes of the microorganisms and the negative functional groups exhibited by the extra-cellular polymeric substances (EPS).

Further evidence of the presence of active biofilm was seen during phase two. During these experiments, the treatment with dense biofilm growth had much higher pH values than the control treatment without biofilm. Differences in pH can be seen after only 2 hours in the mussel shell experiments and after 4 hours in the recycled glass experiments. For example, in the mussel shell experiments, on average, the pH of the biofilm treatment was 2 standard pH units greater than the control treatment after 2 hours. After 4 hours in the recycled glass experiments the difference between the biofilm and the control treatments was as much as 1 standard pH unit (on average; Table 4-10 and Appendix B Table B-3 for more data). Furthermore, the pH results from the batch adsorption experiments (henceforth adsorption experiments), had no biofilm growth and did not reach high pH values as those seen in the phase two biofilm treatments.

This pH trend may be due to the phototrophic nature of the dense biofilm in phase two. Photosynthesis causes changes to the pH of a system as a result of carbon dioxide (CO_2) uptake and release. In particular, it causes increases in the concentration of hydroxyl ions in a solution. When bicarbonate (HCO_3^-) is consumed and the concentration of hydroxyl ions increases, so does the pH of a system (Hu et al., 2005; Beck et al., 2011; Feder, 2014). Interestingly, high pH is known to increase the removal of dissolved heavy metals (e.g. Blecken et al., 2011); however, this was not demonstrated in the present study.

5.1.2 Summary

A dense freshwater autotrophic biofilm with heterogeneous microcolonies was grown in phase two of this study. Filamentous blue-green algae (Cyanobacteria), green algae (Chlorophyta), diatoms (Bacillariophyceae), and bacteria dominated its composition. At first glance, it would appear that the presence of biofilm did not add to the removal of dissolved zinc, copper, and lead from a synthetic stormwater containing typical concentrations of dissolved heavy metals. However, this was not the case, since results from the digestion of the biofilm showed that the biofilm contained high concentrations of heavy metals, with equally high enrichment factors. Furthermore, by making comparisons between the pH of control and biofilm columns, it became clear that the biofilm was an active part of the system and

the difference in removal efficiencies between biofilm and control treatments may be primarily attributed to the influence of the substrate.

5.2 Gravel and Alternative Substrate Efficiency

Gravel is a common construction material frequently used in stormwater treatment systems, while recycled glass and mussel shell are two alternative substrates that have showed potential for their use in stormwater treatment systems. Their affect on dissolved heavy metals concentrations was investigated and all substrates showed excellent dissolved heavy metal removal abilities.

Three distinct patterns in removal efficiency can be seen as a result of the influence of the substrate on dissolved heavy metal concentrations.

1) The long-term experiments (phase one – layered biofilm experiments) in the recycled glass and mussel shell substrates showed that heavy metal removal occurred quickly, with maximum removal occurring within the first 24 hours. This was particularly evident in the mussel shell experiments, in which the removal efficiency of zinc, copper, and lead achieved over 90% within the first 24 hours and remained at this level for the duration of the experiment (168 hours). This result suggested that long exposure times were unnecessary for significant heavy metal removal.

2) Short-term experiments (phase two – whole column biofilm experiments, gravel experiments, and batch adsorption experiments) confirmed that heavy metal removal occurred quickly. Such that the removal efficiency of dissolved heavy metals typically increased over the initial 24 hours, but after 24 hours did not further increase notably. For example, in the recycled glass systems, the removal efficiency of lead increased by 38% between 2 and 24 hours, however, between 24 and 48 hours the increase was less than 1%. A similar trend was seen with copper.

3) All substrates displayed differing affinities for particular heavy metals (Figure 5-1). Mussel shell and gravel substrates achieved identical zinc and copper removal efficiencies, however, mussel shell displayed a lower affinity for copper compared to gravel. Furthermore, gravel achieved its removal efficiencies much sooner than mussel shell, generally within the first 4 hours when compared to the mussel shell which took as long as 12 hours. Recycled glass typically performed intermediate to the mussel shell and gravel, for all three substrates.

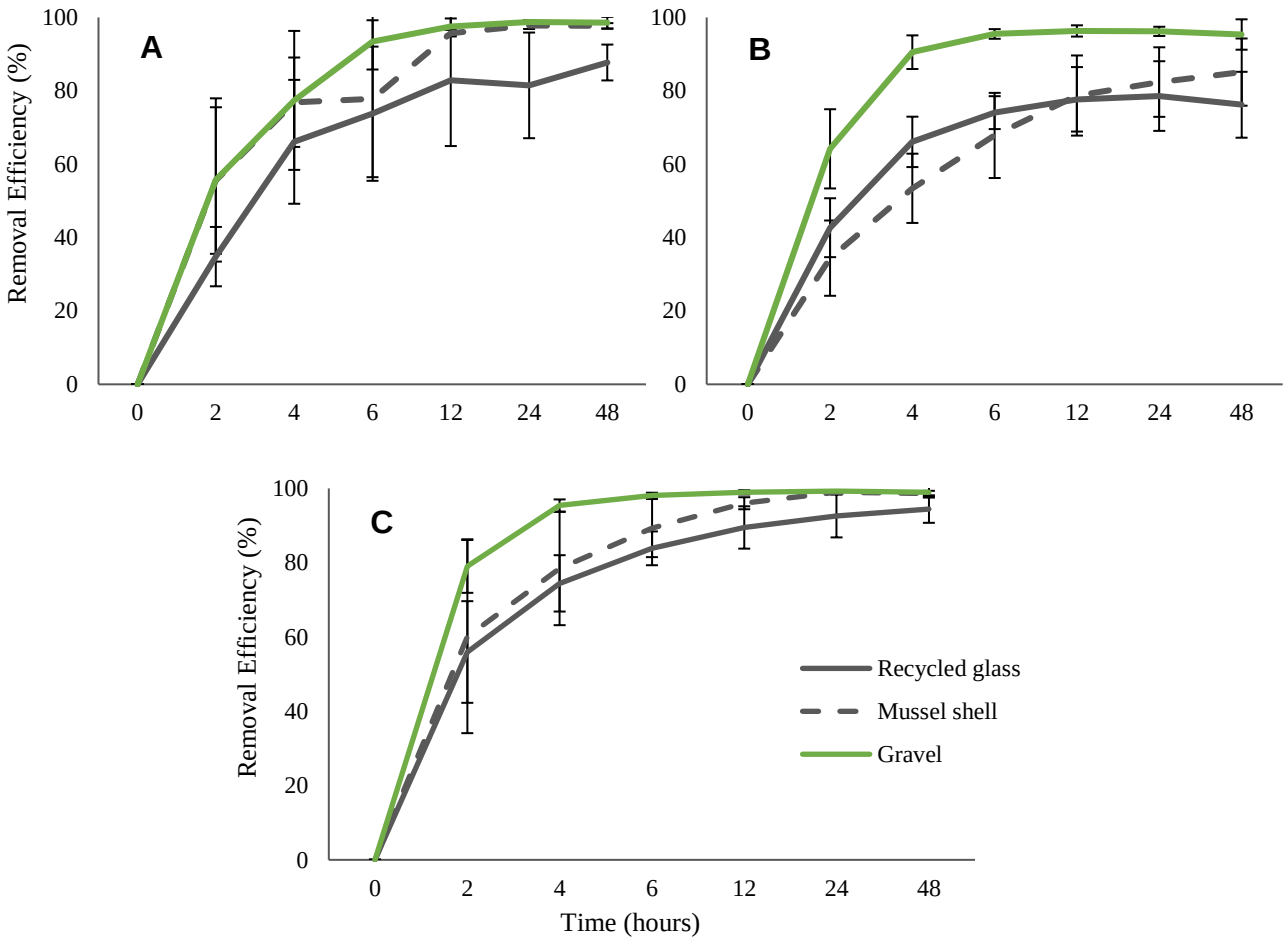

Figure 5-1. The removal efficiency (%) of each substrate for (A) zinc, (B) copper, and (C) lead. Graphs are the average results of phase two experiments, gravel experiments, and the adsorption experiments.

5.2.1 Gravel

Gravel is an abundant material often utilised in stormwater treatment systems. The addition of gravel in these systems is typically because of its drainage and construction properties with very little consideration given to its contaminant removal capabilities. This may be due to the assumption that gravel is an inert substrate and thus very little information on the treatment abilities of gravel is available.

Greywacke, derived from the erosion of the Southern Alps, is abundant on the Canterbury Plains and is the most commonly utilised gravel in stormwater treatment systems in Canterbury. New Zealand greywacke is a hard sandstone which contains quartz and feldspar, held together in a clay-fine matrix (Hodder et al., 1991). This composition is supported by the energy-dispersive x-ray spectroscopy (EDS) results (Table 4-3) which found similar concentrations of oxygen and silicon (quartz) as well as aluminium, potassium, iron, and sodium (potential evidence of feldspar).

5.2.1.1 Heavy metal removal

Gravel achieved excellent removal of dissolved heavy metals from the synthetic stormwater (Figure 5-1), with an order of efficiency of Pb (99%) ≥ Zn (99%) > Cu (96%) after 24 hours (average of the gravel and adsorption experiment results, section 4.6 and section 4.7 respectively). Interestingly, all three heavy metals followed similar removal patterns (Figure 4-12) reaching maximum removal efficiencies at approximately 12 hours. After which lead continued to slightly increase in removal efficiency while zinc and copper showed slightly decreasing trends. These results suggest that the gravel used in this study has characteristics, such as mineral composition and surface area, which provide heavy metal removal capabilities not previously known about.

In the past, the contaminant removal capabilities of stormwater treatment systems have been attributed to the composition of filter media used (Fassman et al., 2013; Lim et al., 2015) which typically consists of sand, topsoil, and organic matter. However, results from the present study, as well as Norris et al. (2013) and Hatt et al. (2007) support the view that gravel has good heavy metal removal abilities. Research by Norris et al. (2013) used a local microgabbro gravel with a mineralogy predominantly consisting of plagioclase feldspar and quartz to investigate the performance of three variations of the microgabbro gravel, namely: unrinsed gravel (dust and particulate material remained on the surface of the gravel), rinsed gravel, and scrubbed gravel (material was scrubbed with a brush so that no dust or particulate material remained on the surface). Their results showed that all microgabbro gravel removed 95 – 100% of dissolved copper and zinc after 48 hours which supports the results of the present study. Interestingly, the scrubbed gravel, which had had its weathered surface removed, showed only 55 – 60% removal after 8 hours compared to the unwashed gravel which showed >80% removal after 8 hours (Norris et al., 2013). Norris et al. (2013) attributed the heavy metal removal abilities of the microgabbro to the dust and particulates, such as clay, found on the surface of the gravel. Clay is known to have very good heavy metal adsorption capabilities (e.g. Zhao et al., 2010; Uddin, 2017) and hence it was hypothesised by Norris et al. (2013) that clay dust and particulates had weathered from the microgabbro itself and were acting as a natural enhancement to the gravel.

A comparable situation may be occurring with the New Zealand greywacke gravel used in this study given that it contains proportions of montmorillonitic clay minerals and feldspar which transforms to the clay mineral kaolinite during weathering (Hodder et al., 1991). This is further supported by the scanning electron microscope (SEM) images which showed the surface of the gravel to be visibly weathered and not pristine (Figure 4-5); however, it is difficult to be sure without further analysis of the composition of the greywacke and its weathered components. As mentioned previously, clay has excellent heavy metal ion adsorption capabilities. This is due to its negative surface charge and high specific surface area, with the uptake of dissolved heavy metals the result of a series of complex adsorption mechanisms (e.g. direct bonding between the heavy metal ion and the clay surface, surface

complexation, and ion exchange) (Uddin, 2017). Both montmorillonitic clay minerals and kaolinite clays are known to display excellent adsorption capabilities (e.g. Uddin, 2017).

If indeed adsorption is the main heavy metal removal mechanism of the gravel then the pH of the solution will have the biggest affect on the adsorption of heavy metal ions to particulates (e.g. clay) because adsorption is strongly pH dependent (Mosley et al., 2001). For example, Veli et al. (2007) found that the highest removal efficiencies of copper and zinc by a natural clay occurred at a pH of over 6, and more specifically, the optimum pH for copper and zinc adsorption was 7 and 8 respectively. Norris et al. (2013) recorded similar pH values of 8.1 ± 0.2 and 7.9 ± 0.2 during batch experiments of gravel with single metal solutions containing zinc and copper respectively, and in the current study adsorption experiments had pH values of approximately 8.2. However, pH values recorded during the column experiments were considerably lower at 6.7. Furthermore, the relationship between pH and the removal efficiency of gravel is moderate at best (Figure 4-14 C) suggesting that greywacke gravel may not be influenced as significantly by pH as recorded in the literature. Also, it is important to note that the two previously mentioned studies utilised single metal solutions, whereas the current study used multi-metal solutions. This may account for the moderate relationship between the removal efficiency of gravel and pH due to the competitive adsorption behaviour of metal ions in multi-metal solutions (e.g. Mosley et al., 2001; Gülbaz et al., 2015). Furthermore, Veli et al. (2007) aptly noted that the affect of pH can differ depending on the substrate (adsorbent surface), its behaviour in solution, and the type of heavy metal ions.

5.2.1.2 Summary

Gravel is a common construction material used extensively in stormwater treatment systems. The results of this research have shown greywacke gravel, which is abundant in New Zealand, to be excellent at the removal of dissolved heavy metals. The excellent removal ability may be attributed to the high clay content within the greywacke gravel, as clay is known to have excellent adsorption properties. The weathered surface of the gravel, the pH values mentioned and the lack of relationship between pH and heavy metal removal efficiency would further support adsorption as the main removal mechanism. This is because other removal mechanisms (e.g. precipitation) are dependent on higher pH values than recorded in this study. Based on these results, the role of gravel in stormwater treatment systems should be reconsidered in the future to take advantage of its excellent dissolved heavy metal removal potential.

5.2.2 Recycled Glass

Recycled glass is a municipal waste product that was originally included in this study as an inert control substrate that would not influence the growth of biofilm. However, results showed that the recycled glass does effectively treat dissolved heavy metals in a laboratory setting (Figure 5-1), with a removal efficiency order of Pb (96%) > Zn (86%) > Cu (73%) after 24 hours exposure (average of all control recycled glass experiments at 24 hours).

5.2.2.1 Substrate characterisation

SEM images of the recycled glass showed variation in the size and shape of the recycled glass particles, with surfaces that were smooth and angular with some particulate material. These visual observations are consistent with sphericity (the shape and its similarities with a sphere) values of 0.41 – 0.43 reported by Soyer et al. (2013), compared to sand, which is much less angular than the recycled glass, and typically has a sphericity value between 0.70 – 0.76 (Soyer et al., 2013). EDS results (Table 4-3) revealed the chemical composition of the recycled glass to be dominated by silicon (33 Wt%) and sodium (10 Wt%), with small amounts of calcium (5 Wt%) and magnesium (3 Wt%). Ibrahim et al. (2012) described a similar chemical composition of recycled glass which was dominated by identical elements but differing in Wt%, as follows: silicon (70 Wt%), sodium (14 Wt%), calcium (11 Wt%), and magnesium (3 Wt%).

Results from the characterisation of the recycled glass showed a very high hydraulic conductivity of 37.4 m/hr whereas the hydraulic conductivity reported in the literature was much lower at 0.1 m/hr (Disfani et al., 2012). Regardless, the hydraulic conductivities mentioned above easily meet the minimum conductivity of 30 mm/hr (or 0.03 m/hr) stipulated by the Christchurch City Council (CCC, 2016). Given that the hydraulic conductivity of the recycled glass was high, its porosity was also measured, as an alternative parameter, for comparison with other bioretention substrates. The recycled glass had a porosity of 42% which is slightly lower than that reported in other studies. For example, Soyer et al. (2013) measured the porosity of crushed glass at 49%. However, it is at the top end of porosities measured for typical stormwater treatment system substrates (Table 5-1). This is unsurprising given that recycled glass is a highly angular substrate that could be considered more free-draining than other natural aggregates utilised in stormwater treatment systems (Disfani et al., 2012). This is a crucial element as porosity can have major implications on the ability of the recycled glass to filter particulates (Soyer et al., 2013).

Table 5-1. Porosity ranges for typical stormwater treatment system substrates as well as recycled glass and mussel shell (Fetter, 2000).

Material	Porosity range (%)
Well-sorted sand or gravel	25 – 30
Mixed sand and gravel	20 – 35
Silt	35 – 50
Clay	33 – 60
Recycled glass	40 – 50
Mussel shell	50 – 70

5.2.2.2 Heavy metal removal

Long-term control experiments (phase one) using recycled glass showed that the substrate was capable of removing high concentrations of dissolved heavy metals quickly, typically within the first 24 hours of exposure. In the case of all three heavy metals, after an initial peak in heavy metal removal at 24 hours for the remainder of the experiment (168 hours) the removal efficiency of the glass decreased. This trend was seen most significantly for zinc and copper. Lead achieved the highest removal efficiency within the recycled glass treatment. The short-term control (phase two) and adsorption experiments supported this result and revealed the effect of the recycled glass on removal efficiency in more detail.

Recycled glass removed zinc to low concentrations, however, there were discrepancies between the three experiment types. As mentioned above, in the long-term experiments zinc displayed an initially high removal efficiency followed by a substantial decrease over the remainder of the experiment. Which would suggest that an increased exposure time of zinc to the recycled glass does not increase the removal efficiency and that just 24 hours exposure can result in efficient removal of zinc (Figure 4-6 A). However, the results of the short-term and adsorption experiments did not support this. The results of the short-term experiments would suggest a minimum of 24 hours exposure to ensure high removal rates of dissolved zinc by the recycled glass (Figure 4-9 A). But in contrast to the long-term experiments, maximum removal efficiency could be gained by increasing exposure time to 48 hours. The adsorption experiments showed a similar trend whereby dissolved zinc was removed quickly, reaching a maximum removal efficiency after 12 hours (Figure 4-13 A).

Dissolved copper had the lowest removal efficiency of the three heavy metals when exposed to recycled glass. Long-term experiments showed that as with zinc the longer exposure time to the recycled glass did not increase the removal efficiency of dissolved copper, such that a maximum removal efficiency occurred after 24 hours exposure. Short-term and adsorption experiments supported this observation because both displayed a steady increase in removal efficiency up to 24 hours exposure, after which

there was only a slight increase in removal efficiency in either experiment (Figure 4-10 A and Figure 4-13 B respectively).

Lead consistently achieved the highest removal in all experiments using recycled glass, achieving over 90% removal efficiency. As with zinc and copper in the long-term experiments, dissolved lead also showed an initial peak in removal efficiency at 24 hours followed by a decrease of approximately 3%. Results from the short-term and adsorption experiments differed slightly. Short-term experiments showed that lead was steadily removed from the synthetic stormwater before reaching a maximum removal efficiency of 97% at 24 hours (Figure 4-11 A). Whereas, the results from the adsorption experiments showed a high removal efficiency of 72% after just two hours exposure to the recycled glass, followed by another increase until the removal efficiency levelled out at 95% after 12 hours exposure (Figure 4-13 C).

Few studies have investigated the use of recycled glass for the treatment of stormwater, in particular, the removal of dissolved heavy metals in stormwater. However, the few that do show the removal of heavy metals by recycled glass. For example, Seelsaen et al. (2006) investigated two different sized fractions of recycled glass for the treatment of zinc and copper (exact size fractions were not specified by the authors). After 24 hours exposure to the recycled glass, the fine glass fraction had a removal efficiency of 69% and 39% for zinc and copper respectively; the coarse glass fraction, on the other hand, displayed much lower removal efficiencies of 16% and 26% for zinc and copper respectively (Seelsaen et al., 2006). Meanwhile, Petrella et al. (2009), recognised an additional advantage of recycled glass in that it successfully removed lead ions from wastewater. Interestingly, both studies identified different removal mechanisms utilised by the recycled glass for the removal of heavy metals from solution. Petrella et al. (2009) identified adsorption, specifically ion exchange, as the key removal mechanism for lead ion removal due to the equivalent release of sodium ions to the liquid phase which were detected in the effluent. However, Seelsaen et al. (2006) attributed the removal of zinc and copper ions to precipitation which was greater in the fine glass fraction due to higher dissolved organic carbon (DOC) leaching rates. This is because DOC can change the solution pH thus increasing the precipitation of heavy metal ions from a solution. At this point it is important to note that the recycled glass used by Petrella et al. (2009) in their research was porous. Recycled porous waste glass, as they call it, is the result of the further manufacturing of the raw glass material whereby it is heated and expanding agents are added to create a light and porous structure.

Adsorption is a surface-based process in which dissolved heavy metal ions are transported to the porous surface of an absorbent (the solid on to which the ions are absorbed) by diffusion and are then adsorbed to the extensive surface area of the absorbent (Xu et al., 2017). Therefore, the porous nature of the recycled glass used by Petrella et al. (2009) would support their ion exchange theory. Precipitation, on the other hand, occurs in aqueous solutions when a change in geochemical conditions converts dissolved

heavy metal ions into an insoluble solid phase via a chemical reaction with a precipitant agent (Lewis, 2017). Typically, the heavy metal precipitated from the solution is in the form of a hydroxide but it can also be in the form of chlorides, sulfates or sulfides, and carbonates (Kurniawan et al., 2006; Lewis, 2017). Precipitation is a pH dependent process such that at higher pH values, such as one afforded by the recycled glass (influent pH = 5.5 ± 0.5, average pH across all experiments = 7.9 ± 0.5), the heavy metal ions can change between dissolved and particulate form via precipitation (Mosley et al., 2001). Once in particulate form, the substrate can filter the particulates out of the solution. This is crucial given that stormwater treatment systems typically rely on contaminant removal through the filtration of particulate-bound heavy metals.

The smooth surface of the recycled glass (Figure 4-3) used in the present study supports precipitation as the main heavy metal removal mechanism in the experimental systems. However, the EDS results showed small amounts of potassium and sodium present in the composition of the recycled glass. These ions are known to be exchangeable cations, which are exchangeable with other cations in solutions such as lead and zinc (Erdem et al., 2004) suggesting that ion exchange may be working as a secondary removal mechanism in the present study. The high removal efficiencies of lead and zinc, but not copper, further support this theory.

The poor copper removal efficiency may be attributed to the presence of organic matter within the recycled glass systems. Recycled glass is known to leach DOC (e.g. Seelsaen et al., 2006) and dissolved copper is known to be significantly correlated with DOC concentrations in natural stormwater (Mosley et al., 2001). Copper which has the strongest affinity for organic matter can form stable Cu-DOC complexes with DOC which mobilises the complex into solution, ultimately resulting in higher concentrations of copper in the effluent (Antoniadis et al., 2002; Blecken et al., 2011). Despite higher concentrations of copper being present in the effluent, compared to zinc and lead, it is merely speculation as to the role of DOC in the current study given DOC was not measured in any of the experiments. Furthermore, DOC can influence the pH of an aqueous solution directly because of its acid properties, but also indirectly by affecting the buffer systems regulating pH in an aqueous environment (Erlandsson et al., 2010). As a result, the presence of DOC may influence the precipitation of heavy metals through changes in pH. Regression analyses showed there is effectively no relationship between pH and the removal efficiencies of any of the three heavy metals by the recycled glass (Figure 4-14 A). Despite this, the presence of DOC may go some way to explain the decreasing trends in removal efficiency seen with all three heavy metals in the long-term experiments. This is because DOC can be preferentially adsorbed to the surfaces of solids effectively competing with the heavy metal ions for binding sites, thus reducing metal binding (Antoniadis et al., 2002).

5.2.2.3 Summary

Widely thought to be a chemically inert substrate, recycled glass has shown its potential to treat dissolved heavy metals effectively. From the results of this study, we can speculate as to how the recycled glass provides these heavy metal removal abilities. Precipitation is likely the key heavy metal removal mechanism given the smooth surface of the recycled glass and the high pH values recorded. It is also possible that ion exchange with potassium and sodium is occurring to some degree. However, more research is needed to explore these mechanisms in detail and to narrow down the properties involved. In a wider context, utilising recycled glass as an alkalinity amendment for stormwater treatment in the future would add value to the life cycle of the recycled glass while providing beneficial treatment of the stormwater for the protection of the environment.

5.2.3 Mussel Shell

Mussel shells are an abundant alkaline waste product from the shellfish industry, which may work as a pH buffer in stormwater treatment systems to improve the removal of dissolved heavy metals from stormwater. Few studies have reported on the use of mussel shells for stormwater treatment. In the current study, mussel shell showed excellent dissolved heavy metal removal ability (Figure 5-1), with a removal efficiency in the order of Pb (97%) ≥ Zn (97%) > Cu (89%) after 24 hours exposure (average of all control mussel shell experiments at 24 hours).

5.2.3.1 Substrate characterisation

SEM and EDS results of the mussel shell show a highly porous substrate that is composed predominantly of oxygen (69 Wt%) and calcium (22 Wt%), with a small amount of sulfur (4 Wt%), aluminium (2 Wt%) and silicon (2 Wt%). This composition is comparable to compositions that are reported in the literature for mussel shell (e.g. Abdulkarim et al., 2013).

Results from the hydraulic conductivity and porosity testing, 22 m/hr and 56% respectively, indicated that the crushed mussel shell used in this study could be considered as a well-draining substrate. Furthermore, this conductivity meets the requirements of 33 mm/hr (0.03 m/hr) set out by the Christchurch City Council (CCC, 2016). Weber et al. (2015) also reported good hydraulic conductivity of 3.6 m/hr for mussel shell as well as a porosity of 72% which is much higher than that recorded during this study and also the porosities reported for common stormwater treatment system substrates (Table 5-1). However, it is difficult to make comparisons between the conductivity and porosity results of the mussel shell reported in this study and those reported in other studies. This is because the size fraction used in other studies was often different to the fraction used in this study. For example, often the mussel shell is only roughly crushed before use which would support the much higher porosity reported by

Weber et al. (2015). Whereas, in the current study a specific size faction was tested that resulted in different hydraulic conductivity and porosity measurements when compared to the literature.

5.2.3.2 Heavy metal removal

Heavy metal removal efficiencies were consistent across all mussel shell experiments. During the long-term control treatment experiments, maximum dissolved heavy metal removal was typically achieved after 24 to 48 hours of exposure for all three dissolved heavy metals. For all heavy metals tested (zinc, copper, and lead) some variation over time was seen in removal efficiencies, however, for all heavy metals over 90% removal efficiency was achieved (phase one). Short-term control treatment experiments (phase two) produced consistent removal efficiencies which supported the results of the long-term experiments. For all three heavy metals, the maximum removal efficiency (99.1, 97.1, and 99.0% for zinc, copper, and lead respectively) was reached after 24 hours exposure to the mussel shell. Interestingly, the adsorption experiments showed slightly different results, in which zinc and lead effectively reached their maximum removal efficiencies after just 2 hours exposure to the mussel shell, which was much faster than the short-term experiments. This was particularly obvious for lead which had an increase of approximately 3% between 2 (96%) and 24 (99%) hours (Figure 4-13 C). Copper, on the other hand, showed a gradual increase in the removal efficiency for the duration of the experiment and reached a maximum of just 77% after 24 hours which increased slightly, to 83%, after 48 hours (Figure 4-13 B). This result for copper was somewhat less than the removal efficiencies recorded during the short- and long-term experiments.

Previous studies have demonstrated the usefulness of mussel shell as a pH amendment for the treatment of acid mine drainage (AMD) and stormwater (e.g. Daubert et al., 2007; McCauley et al., 2009; Good et al., 2014). A key study by Good et al. (2014) incorporated mussel shell into the substrate of bioretention systems for the enhanced treatment of dissolved heavy metals in stormwater. Within their experimental systems the mussel shell significantly increased the heavy metal removal efficiency of the systems; for example, the removal efficiency of zinc increased from 55% to 80% in the presence of mussel shell, and copper saw a similar increase from 27% to 47% (Good et al., 2014). However, lead saw no significant difference in removal efficiency in the presence of mussel shell; given that lead is most prevalent in a particulate state it is consistently removed by stormwater treatment systems without the need for pH amendments. Additionally, Good et al. (2014) found that the systems with mussel shell amendments had lower dissolved heavy metal fractions which they attributed to the increased pH caused by the mussel shell. This is because dissolved heavy metal ions shift to predominantly particulate-bound forms at higher pH values (Dempsey et al., 1993). The current study presented much greater removal efficiencies for zinc and copper and equally high lead removal that may be attributed to the lack of any other substrate in the experimental systems, which would add further variables to be considered (e.g. Li et al., 2008).

Studies by McCauley et al. (2009) and Daubert et al. (2007) have both shown the benefit of using mussel shell for the treatment of heavy metals in AMD. For example, in the study by Daubert et al. (2007), they recorded a decrease in dissolved iron, aluminium, and manganese concentrations as pH increased in the presence of chitin derived from crab shell. Such that the concentration of dissolved iron and aluminium dropped to less than 0.03% of the starting concentration, thus demonstrating the significant contaminant removal abilities of chitin (Daubert et al., 2007). McCauley et al. (2009) supported this view. Using mussel shell McCauley et al. (2009) found that the systems with the most mussel shell showed the best heavy metal (iron and aluminium) removal.

In all of the studies mentioned above, heavy metal removal was attributed to both adsorption and precipitation (and ultimately filtration), and it is likely that either or both processes are being utilised in the current study. The composition of the mussel shell facilitates both adsorption and precipitation processes for dissolved heavy metal removal. Mussel shell is predominantly composed of minerals in the form of calcium carbonate ($CaCO_3$; 52%) and chitin (38%) (Abdulkarim et al., 2013). EDS results showed that the mussel shell used in this study had a similar composition to the other studies with high concentrations of oxygen and calcium measured.

Chitin is a natural biopolymer which has excellent adsorption potential because it contains high concentrations of amino and hydroxyl functional groups (Daubert et al., 2007; Bhatnagar et al., 2009). These functional groups are highly reactive enhancing the ability of chitin to adsorb heavy metals; furthermore, the adsorption of heavy metals by the chitin is pH dependent, with adsorption enhanced up to a pH of 7 (Bhatnagar et al., 2009). Chitin also has an order of affinity for heavy metal ions as follows: copper > lead > zinc (Bhatnagar et al., 2009). The second component of mussel shell is $CaCO_3$. The dissolution of the $CaCO_3$ component of the mussel shell likely results in increased alkalinity subsequently resulting in increased pH (Daubert et al., 2007; McCauley et al., 2009; Good et al., 2014). Alkalinity, and to a lesser extent pH, were considerably higher in the mussel shell systems than in either the gravel or recycled glass systems, with an average alkalinity of 195.5 ± 55.8 mg/L as CaCO3 (average of phase one and two control treatments). Similar alkalinities have been recorded in the literature (e.g. Daubert et al., 2007). When pH reaches a value above 7 precipitation of the heavy metal ions occurs, after which the substrate simply filters out the newly particulate metals (Dempsey et al., 1993). Therefore, we can speculate that at a pH value below 7 adsorption will be the predominant dissolved heavy metal removal mechanism, while at pH values above 7 precipitation may dominate as the key removal mechanism.

Given that pH is an important physicochemical parameter controlling heavy metal removal in the mussel shell systems, it was surprising to see the significant negative relationships between pH and the removal efficiencies of copper and zinc. Regression analyses showed a significant negative relationship between the removal efficiency of copper and pH ($n = 12$, $P < 0.000$), showing that as the pH in the

mussel shell increased during the adsorption experiments the removal efficiency of copper decreased. Zinc showed a similar trend, however, the negative trend was only slight ($n = 11$, $p < 0.000$); and no trend between the removal efficiency of lead by the mussel shell and pH can be seen ($n = 12$, $p < 0.000$). It is important to note that the regression analyses discussed here are based on the adsorption experiments only. However, they would suggest that in the case of zinc and copper there is a negative relationship between removal efficiency and pH, while no relationship is present between the removal efficiency of lead and pH. Given that lead is predominantly associated with particulates regardless of pH, this relationship may be expected. The relationship between copper and zinc and pH is much harder to explain, however, it is noted that during the adsorption experiments the pH was initially high (pH at hour 2 = 8.4) and decreased with time (pH at hour 48 = 7.7). By looking at the results of phase two experiments we can see that a maximum removal efficiency was not achieved until after 12 to 24 hours exposure of the synthetic stormwater to the mussel shell. Therefore, in the adsorption experiments pH dropped while removal efficiency was still increasing, such that higher removal efficiencies were recorded when the pH was lower which may not be indicative of what was happening in the other systems. Why the pH of the mussel shell decreased over time in the adsorption experiments is unknown and would require further investigation.

5.2.3.3 Summary

Overall, the mussel shell showed potential as a pH buffering substrate within stormwater treatment systems to improve the removal of dissolved heavy metals. Results from this study showed that the mussel shell was capable of removing high concentrations of dissolved heavy metals from a synthetic stormwater. Both adsorption and precipitation are likely removal mechanisms due to the composition of the mussel shell. Firstly, dissolution of $CaCO_3$ in the mussel shell results in increased alkalinity and pH, which in turn supports the precipitation of heavy metal ions from the solution. Secondly, mussel shell is largely composed of chitin which contains large quantities of amino and hydroxyl functional groups giving mussel shell excellent adsorption potential. As with the recycled glass, utilising mussel shell would reduce waste produced by the shellfish industry while adding value to the lifespan of the mussel shell. However, more research is needed to understand the affect of mussel shell in the field as well as its lifespan as a buffering material in field situations.

Chapter 6. Conclusions and Future Research Recommendations

6.1 Conclusions

The overall aim of this research was to assess whether the growth of biofilm on a substrate could improve the removal of dissolved heavy metals from stormwater treatment systems. This aim evolved with the discovery that the recycled glass and gravel, used as a control substrate for the original experiments, showed excellent heavy metal removal abilities on their own. Such that the objectives of this study grew to allow the author to evaluate the dissolved heavy metal removal abilities of gravel, recycled glass, and mussel shell.

The main objectives of this research were:

1. To quantify how effectively biofilms sorb dissolved heavy metals from stormwater over time;
2. To determine if biofilm adsorb dissolved heavy metals from a synthetic stormwater to a greater extent than substrate alone;
3. To determine the efficiency of recycled glass, mussel shell, and gravel substrates to remove dissolved heavy metals from a synthetic stormwater;
4. To investigate the dissolved heavy metal removal mechanism(s) of recycled glass, mussel shell, and gravel.

Objective 1: The effectiveness of biofilm to sorb dissolved heavy metals from stormwater over time.

Biofilm are collections of microorganisms attached to moist abiotic surfaces and enclosed in a matrix of extra cellular polymeric substances (EPS). Biofilms possess a range of properties that make them highly effective at removing dissolved heavy metals from metal-contaminated water (Feder et al., 2015). Together the EPS and the microorganisms within the biofilm can facilitate the sorption of heavy metals, through passive (biosorption) and active (bioaccumulation) cellular processes.

The freshwater biofilm grown in this study sorbed high concentrations of dissolved heavy metal from their environment, which was evident from high enrichment factors. This ability of biofilms had been demonstrated in a range of environments from wastewater and stormwater (e.g. Costley et al., 2001; Feder et al., 2015) to freshwater (e.g. Farag et al., 2007; Ancion et al., 2010; Ancion et al., 2013). A range of active and passive removal mechanisms can be attributed to the removal of dissolved heavy metals within aqueous environments.

Unfortunately, the initial concentrations of heavy metals within the biofilm were not measured, but initial biofilm growth occurred without known sources of heavy metal contributions and initial concentrations of heavy metals were assumed to be negligible. Therefore, the extent to which biofilms

are capable of sorbing dissolved heavy metals from an aqueous solution cannot be fully quantified. However, it is unlikely that the high concentrations of heavy metals measured in the biofilm are from background sources alone, especially since the concentrations recorded are typically greater than those recorded in the literature.

Objective 2: Do biofilms adsorb dissolved heavy metals from a synthetic stormwater to a greater extent than substrate alone?

Biofilms did not have a significant impact on dissolved heavy metal removal relative to the substrate on its own, despite biofilms demonstrating an ability to sorb dissolved heavy metals to high concentrations (objective 1). Results suggest that the presence of biofilm may inhibit the removal of dissolved heavy metals in both the recycled glass and mussel shell substrates to a certain degree. In some cases, however, particular combinations of substrate and biofilm did show increased removal efficiencies compared to substrate alone. Both phenomenon whereby the biofilm either increases or inhibits the removal of heavy metals from aqueous solutions has been reported in the literature (e.g. Kulczycki et al., 2005; Anderson et al., 2006; Feder et al., 2015).

Ultimately, during this study, a dense freshwater biofilm was grown on two alternative materials, recycled glass and mussel shell. Comparisons between the control substrate and substrate with biofilm found no noteworthy differences in dissolved heavy metal removal efficiency, from which it can be concluded that the addition of biofilm to a substrate added no heavy metal removal benefit, except in select examples.

Objective 3: The efficiency of recycled glass, mussel shell, and gravel to remove dissolved heavy metals from a synthetic stormwater.

Recycled glass is a safe versatile material, which for a long time has been thought to be inert and thus disposed of readily into landfills leading to a long-term build up in the environment. However, recent research has shown that recycled glass has benefits for wastewater and stormwater treatment, which adds value to the life cycle of the recycled glass. Mussel shells are an abundant, alkaline, waste product from the shellfish industry that has been utilised in wastewater and stormwater treatment as a pH buffer. Gravel, on the other hand, is an abundant substrate which is already commonly used as a construction material in stormwater treatment systems.

Gravel and mussel shell consistently showed the best dissolved heavy metals removal efficiencies, typically achieving over 90% removal for zinc, copper, and lead. Meanwhile, recycled glass achieved very good removal efficiencies, just not to the same extent as the gravel and mussel shell substrates.

All three substrates have shown stormwater treatment potential by effectively reducing the concentration of dissolved heavy metals from a synthetic stormwater. However, it is important to note that the long-term efficiency of these substrates is unknown. Although, it is hoped that these results

encourage an increased interest in the recovery of waste products for the protection of the environment in the future. By incorporating recycled glass and mussel shell into stormwater treatment systems we can contribute to waste minimisation and increased value associated with the extended lifecycle of these products. A comparable situation may occur with gravel. By demonstrating its heavy metal removal abilities, thought must now be given as to the best way to utilise this ability to benefit the environment. However it is crucial to note that the lifetime ability of these substrate to remove heavy metal contaminants is unknown and should be considered also.

Objective 4: Investigate the dissolved heavy metal removal mechanism(s) of recycled glass, mussel shell, and gravel.

Scanning electron microscope (SEM) and energy-dispersive x-ray spectroscopy (EDS) analyses were conducted, on all three substrates, to investigate their respective removal mechanisms.

New Zealand's greywacke gravel, the type used in this research, is a hard sandstone which contains quartz and feldspar held together in a clay-fine matrix (Hodder et al., 1991). These components of greywacke weather to form clay particulates on its surface. This was supported by the SEM images that showed an extensively weathered surface. Clay is known to have excellent adsorption properties which are thought to be the main heavy metal removal mechanisms of the gravel.

Recycled glass is characterised by a smooth surface and concentrations of oxygen, silicon, and sodium, with lesser amounts of calcium and magnesium. An extensive literature search suggested that both adsorption and precipitation of heavy metal ions are responsible for the heavy metal removal ability seen. However, precipitation is likely to be the key heavy metal removal mechanism. This is likely because of the increased pH values recorded and the smooth surface of the recycled glass that would not support adsorption properties.

Both adsorption and precipitation are likely to be occurring in the mussel shell systems due to the composition of the mussel shell. Calcium carbonate ($CaCO_3$) and chitin are the dominant components of mussel shell and each component results in a different removal mechanism. $CaCO_3$ dissolved in an aqueous solution causes alkalinity and pH to increase, which results in precipitation of dissolved heavy metals. Chitin is a natural biopolymer found in large concentrations within mussel shell and contains large quantities of amino and hydroxyl functional groups. These functional groups give the mussel shell excellent adsorption potential. Furthermore, it is likely that the porous surface of the mussel shell benefits the adsorption of heavy metal ions to the mussel shell.

In all substrates, it is likely that both the composition and surface textures influence the removal of dissolved heavy metal from aqueous solutions. Also, it is expected that a combination of removal mechanisms are working to produce the heavy metal removal efficiencies seen in this thesis and further research would be needed to narrow down key mechanisms.

6.2 Future Research Recommendations

The following is a list of modifications to the present study and potential future research that would complement the research presented in this thesis.

Modifications include:

- Performing the experiments with the solution fed through the systems by gravity. To ensure the experiments were comparable, synthetic stormwater was pumped from the bottom up though the systems. However, this is not the direction that natural stormwater would flow in the field. Therefore, it would be interesting to perform the experiments with the solution flowing in the opposite direction.
- Conducting experiments using a natural stormwater, rather than a synthetic stormwater. This would evaluate how the natural complexity of the stormwater may affect the contaminant removal capabilities of each substrate.
- Running a range of experiments with different initial concentrations of heavy metals to see whether an adsorption isotherm could be fitted to the experimental data.
- Using a biofilm grown in a stormwater treatment system. Because the composition of biofilm grown in a treatment system would differ from that of a freshwater biofilm as used in this research.
- Measurement of a wider range of water quality parameters, to ensure no detrimental effects may occur if effluent was discharged into a waterway.

Future Research:

- Perform further laboratory and field experiments with the three materials in full-scale stormwater treatment systems.
- Determine the lifetime performance and buffering capacity of the materials for stormwater treatment.
- Targeted studies to further investigate the heavy metal removal mechanisms of the three substrates and the biofilm.
- Further research is recommended to understand how the hydraulic conductivity and porosity of recycled glass and mussel shell may impact stormwater treatment systems when the substrates are incorporated into the design along with other substrates.

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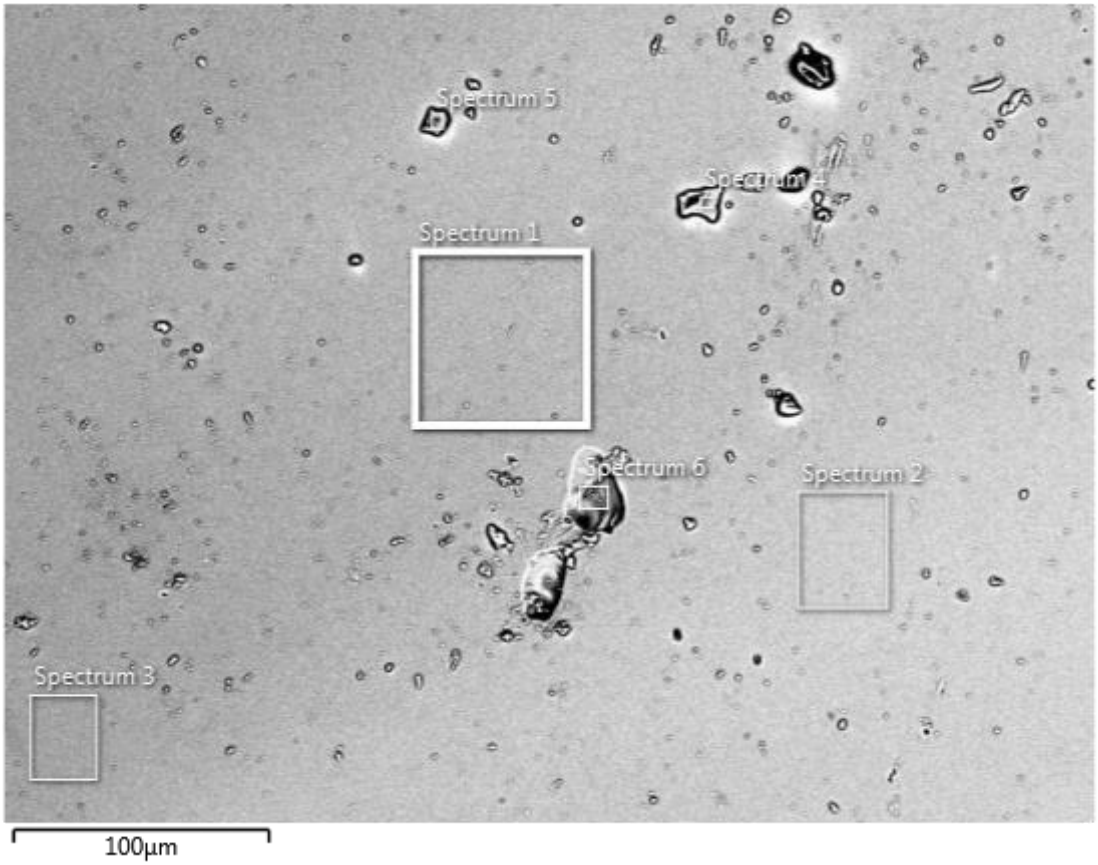
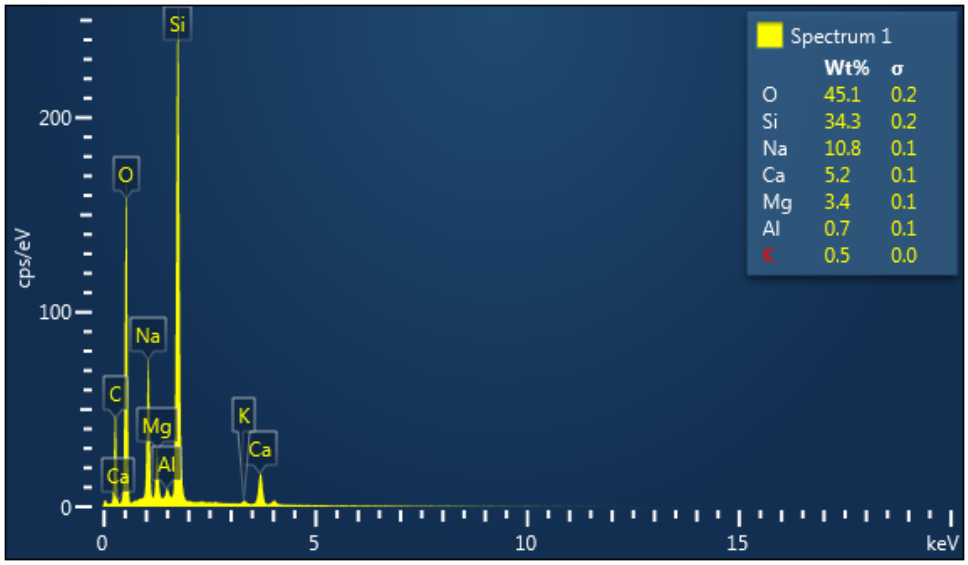
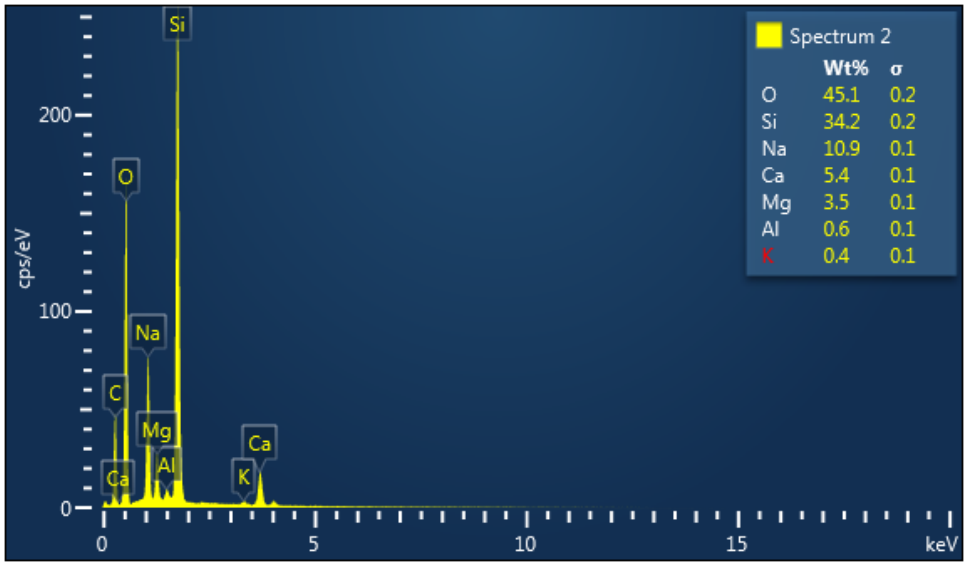
Appendix A – Energy-Dispersive X-ray Spectroscopy Reports

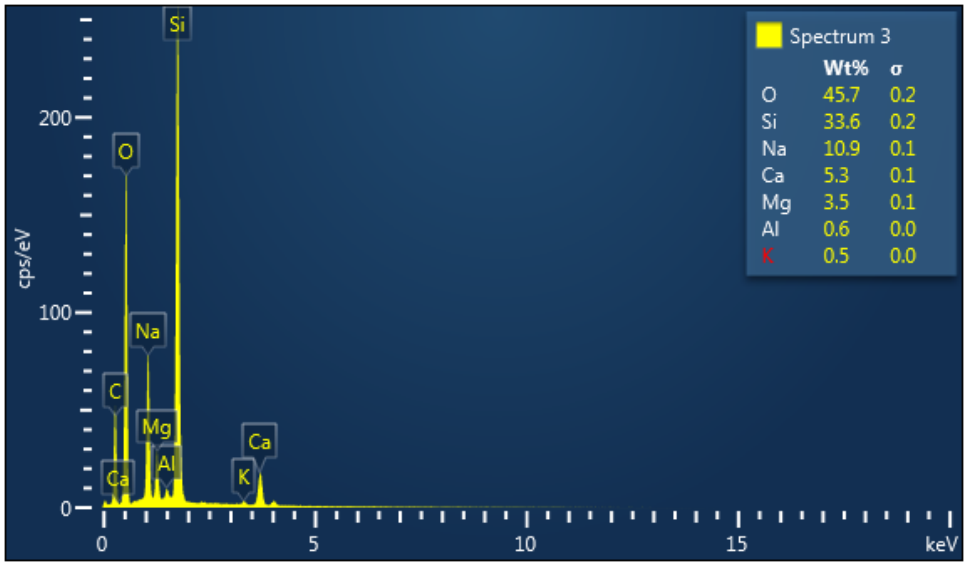
Energy-dispersive x-ray spectroscopy (EDS) analyses were undertaken for all three substrates to determine the chemical composition of the substrates. The following are the full EDS reports generated for each substrate.

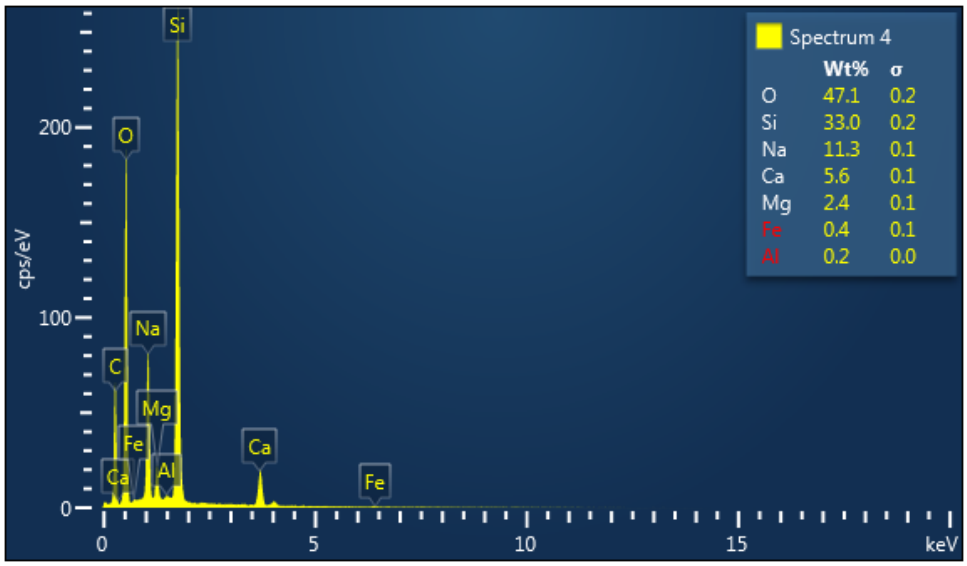
Recycled Glass

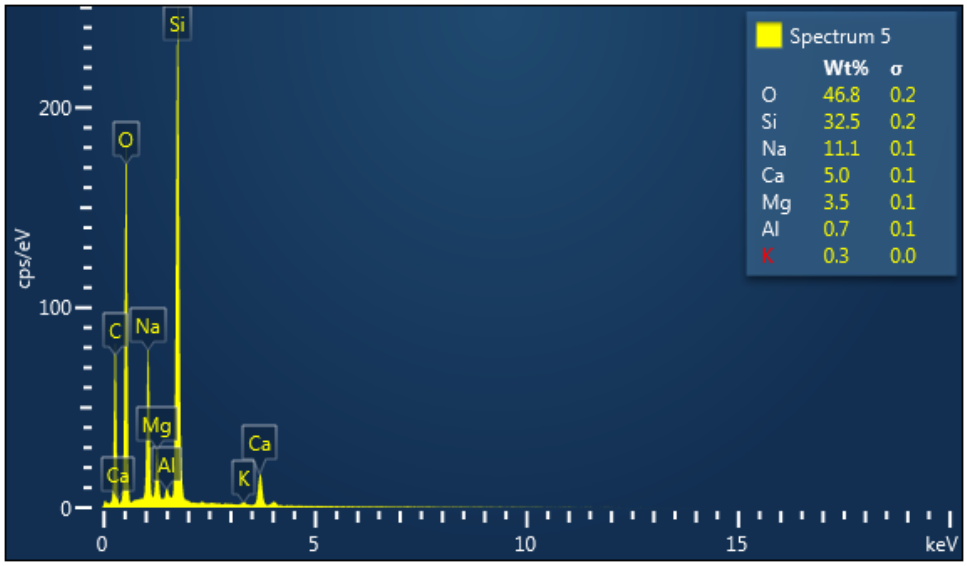



Spectrum 1
Wt% σ
O 45.1 0.2
Si 34.3 0.2
Na 10.8 0.1
Ca 5.2 0.1
Mg 3.4 0.1
Al 0.7 0.1
K 0.5 0.0
cps/eV
keV
Si
O
Na
C
Mg
Al
Ca
K


Spectrum 2
Wt% σ
O 45.1 0.2
Si 34.2 0.2
Na 10.9 0.1
Ca 5.4 0.1
Mg 3.5 0.1
Al 0.6 0.1
K 0.4 0.1
cps/eV
keV
Si
O
Na
C
Mg
Al
Ca
K


Spectrum 3
Wt% σ
O 45.7 0.2
Si 33.6 0.2
Na 10.9 0.1
Ca 5.3 0.1
Mg 3.5 0.1
Al 0.6 0.0
K 0.5 0.0
cps/eV
keV
Si
O
Na
C
Mg
Al
Ca
K


Spectrum 4
Wt% σ
O 47.1 0.2
Si 33.0 0.2
Na 11.3 0.1
Ca 5.6 0.1
Mg 2.4 0.1
Fe 0.4 0.1
Al 0.2 0.0
cps/eV
keV
Si
O
Na
C
Mg
Fe
Ca
Al
Ca
Fe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Wt% σ
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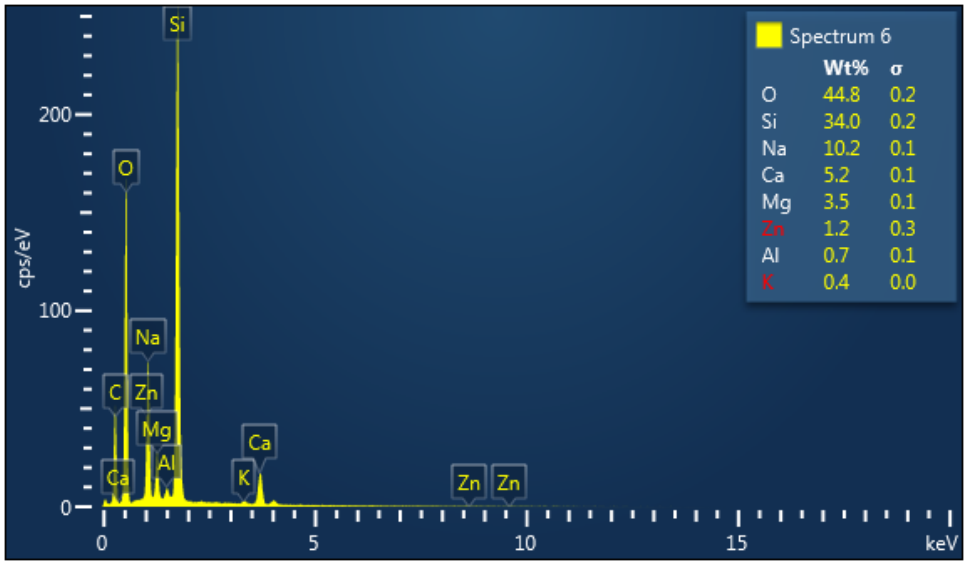
Si 32.5 0.2
Na 11.1 0.1
Ca 5.0 0.1
Mg 3.5 0.1
Al 0.7 0.1
K 0.3 0.0
cps/eV
keV
Si
O
C
Na
Mg
Ca
Al
Ca
K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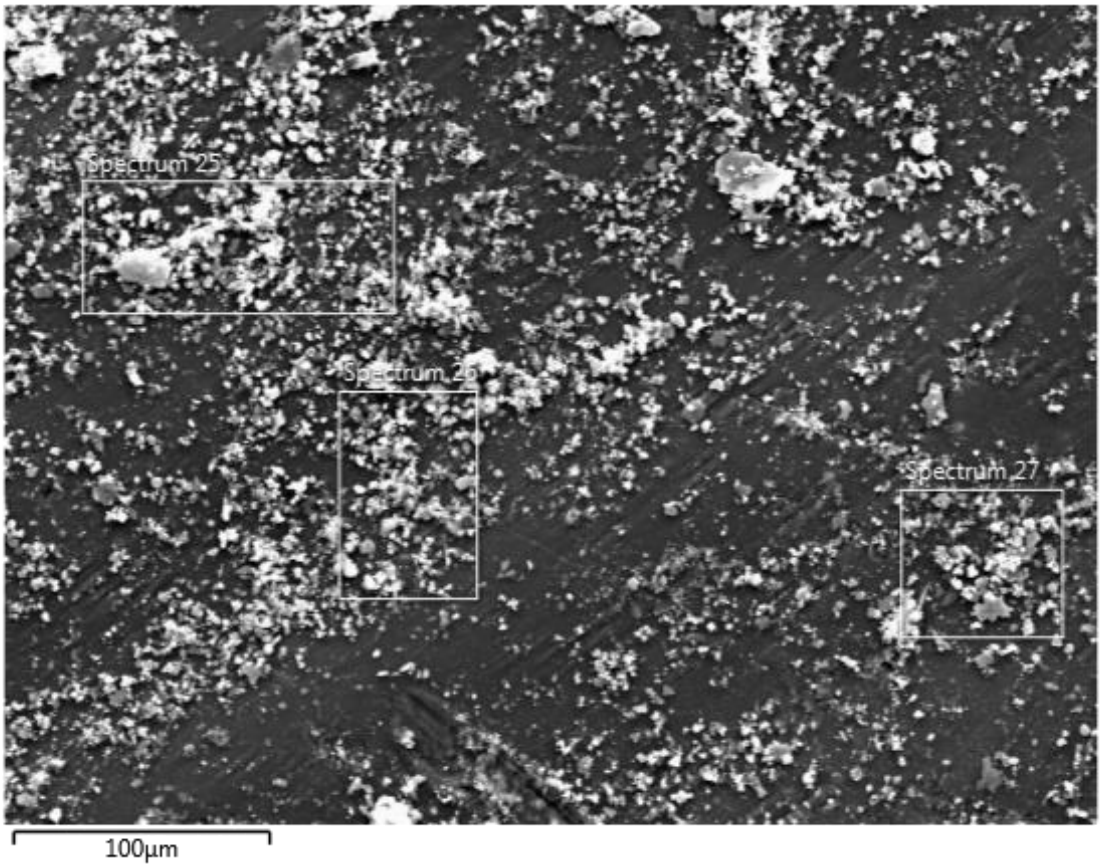
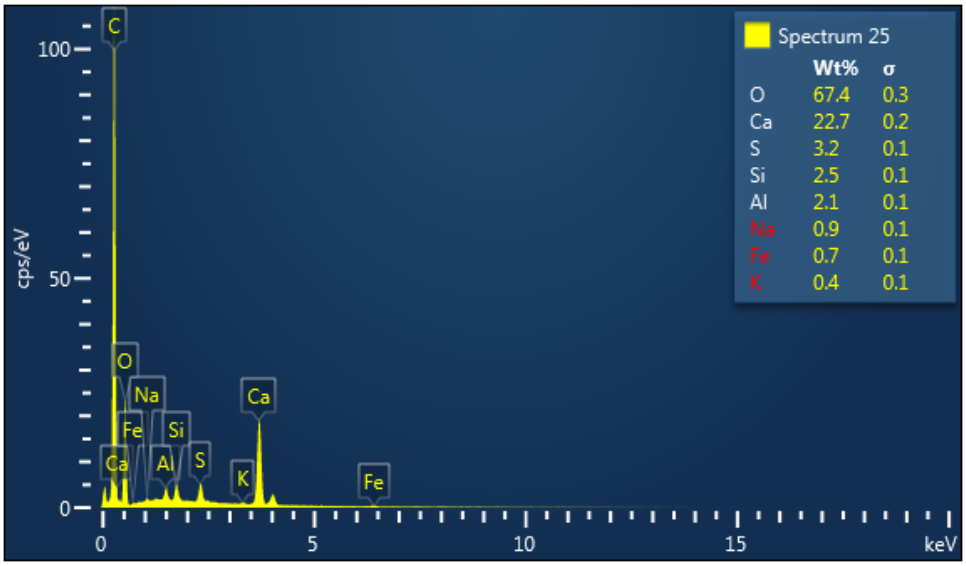
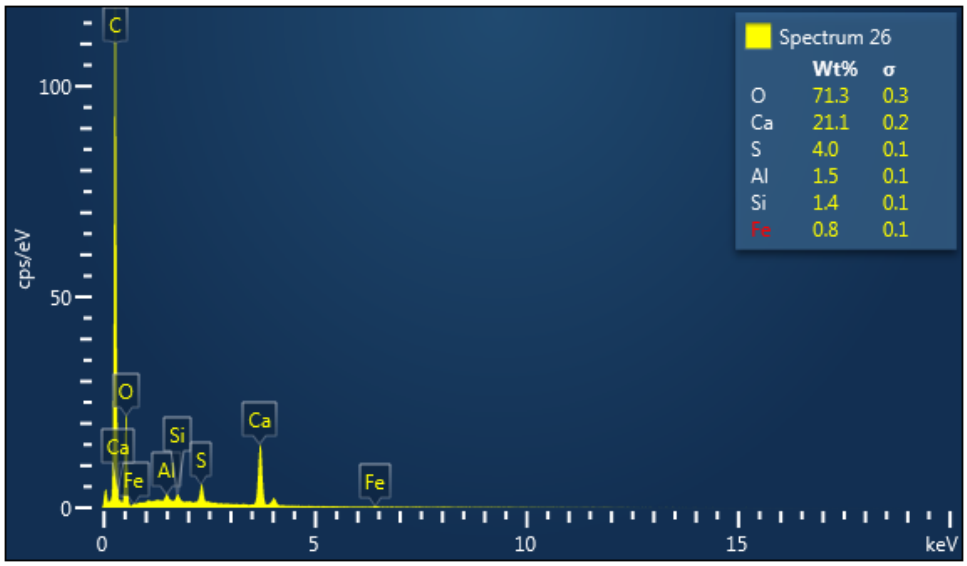
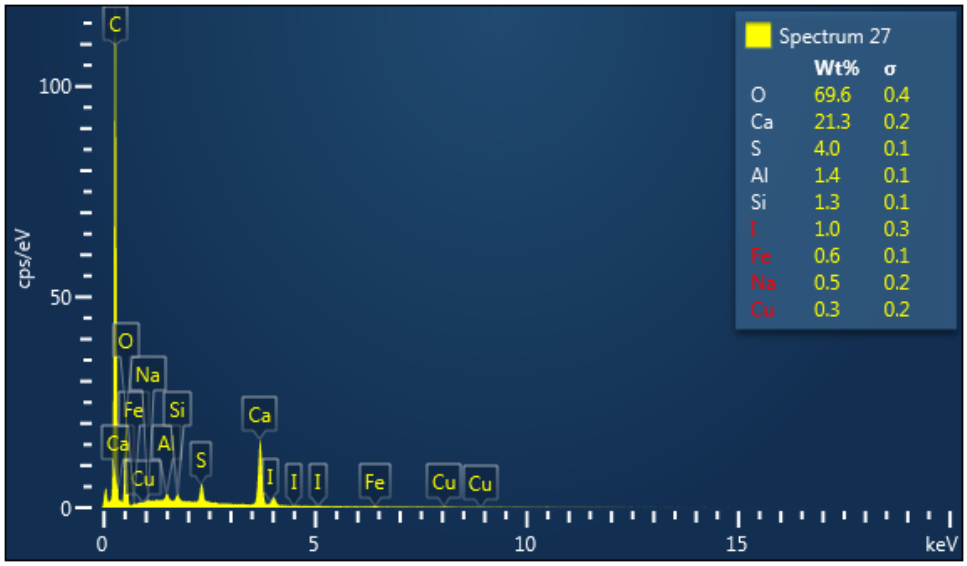
Wt% σ
O 44.8 0.2
Si 34.0 0.2
Na 10.2 0.1
Ca 5.2 0.1
Mg 3.5 0.1
Zn 1.2 0.3
Al 0.7 0.1
K 0.4 0.0
cps/eV
keV
Si
O
Na
C
Zn
Mg
Ca
Al
Ca
K
Zn
Zn

Mussel Shell






Spectrum 26
Wt% σ
O 71.3 0.3
Ca 21.1 0.2
S 4.0 0.1
Al 1.5 0.1
Si 1.4 0.1
Fe 0.8 0.1
cps/eV
keV
C
O
Ca
Fe
Al
Si
S
Ca
Fe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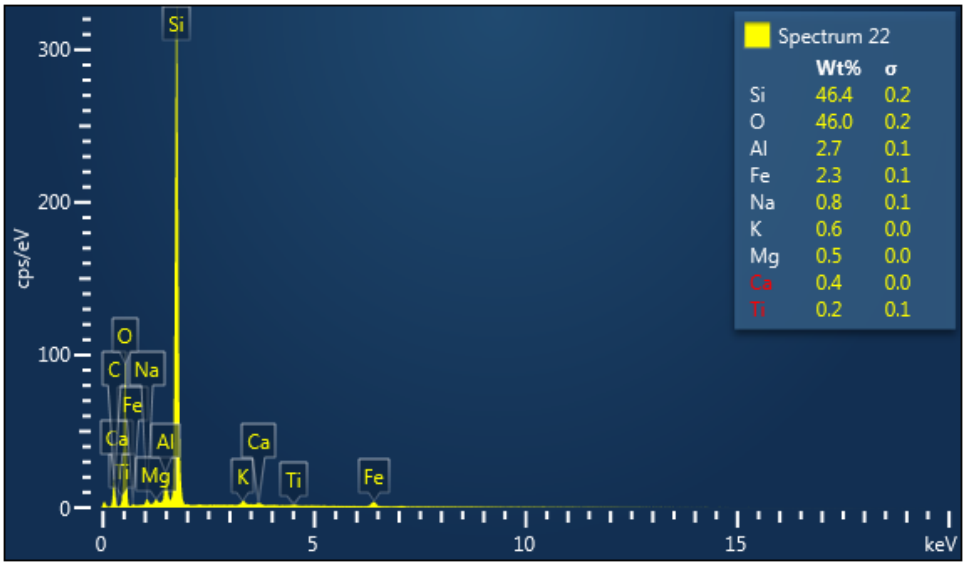
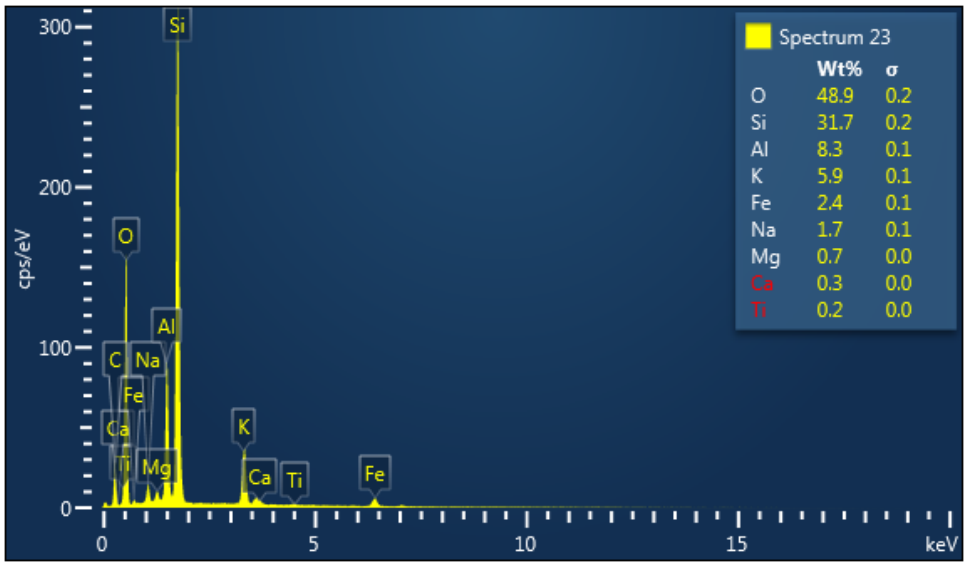
Wt% σ
O 69.6 0.4
Ca 21.3 0.2
S 4.0 0.1
Al 1.4 0.1
Si 1.3 0.1
I 1.0 0.3
Fe 0.6 0.1
Na 0.5 0.2
Cu 0.3 0.2
cps/eV
keV
C
O
Na
Fe
Si
Ca
Ca
Al
S
Cu
I
I
I
Fe
Cu
Cu

Gravel


Spectrum 22
Spectrum 3
Spectrum 21
100µm


Spectrum 22
Wt% σ
Si 46.4 0.2
O 46.0 0.2
Al 2.7 0.1
Fe 2.3 0.1
Na 0.8 0.1
K 0.6 0.0
Mg 0.5 0.0
Ca 0.4 0.0
Ti 0.2 0.1
cps/eV
300
200
100
0
Si
O
C
Na
Fe
Ca
Al
Ti
Mg
Ca
K
Ti
Fe
0
5
10
15
keV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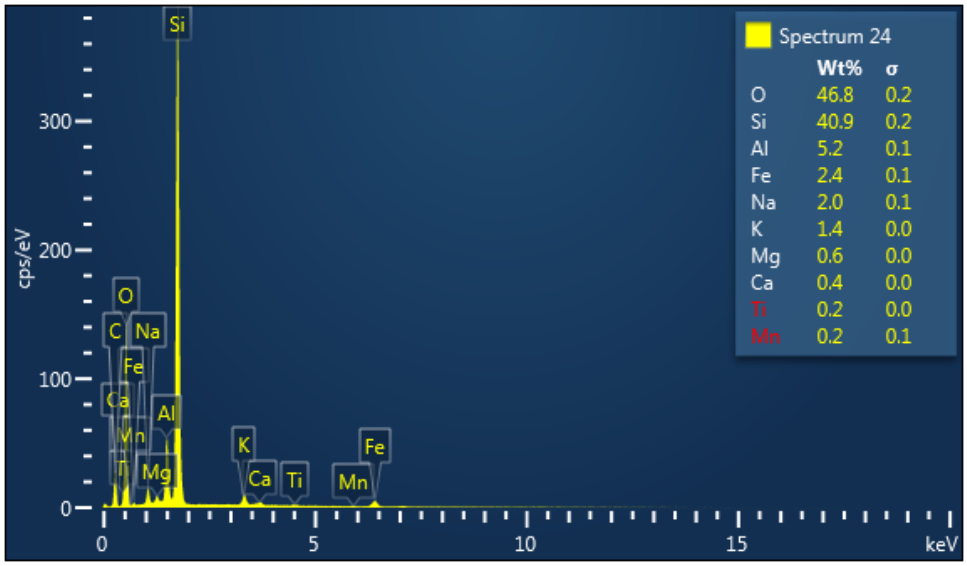
Wt% σ
O 48.9 0.2
Si 31.7 0.2
Al 8.3 0.1
K 5.9 0.1
Fe 2.4 0.1
Na 1.7 0.1
Mg 0.7 0.0
Ca 0.3 0.0
Ti 0.2 0.0
cps/eV
keV
Si
O
Al
C
Na
Fe
Ca
K
Ti
Mg
Ca
Ti
Fe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Wt% σ
O 46.8 0.2
Si 40.9 0.2
Al 5.2 0.1
Fe 2.4 0.1
Na 2.0 0.1
K 1.4 0.0
Mg 0.6 0.0
Ca 0.4 0.0
Ti 0.2 0.0
Mn 0.2 0.1
cps/eV
keV
Si
O
C
Na
Fe
Ca
Al
Mn
K
Fe
Ti
Mg
Ca
Ti
Mn

Appendix B – Water Quality Parameter Data

Phase One – Layered Biofilm Experiments

Table B-1. pH (mean ± SD) measured over 168 hours, including influent (hour = 0), in the layered biofilm experiments for both substrates, treatments have been differentiated as control (no biofilm) and biofilm (all columns with biofilm, either covered or uncovered).

Time (hours)	Recycled glass		Mussel shell	
	Control	Biofilm	Control	Biofilm
0	5.7 ± 1.6	5.7 ± 0.3	5.2 ± 0.3	5.6 ± 0.5
24	8.1 ± 0.5	7.7 ± 0.2	8.2 ± 0.2	8.1 ± 0.1
48	8.1 ± 0.3	7.8 ± 0.2	8.1 ± 0.1	8.1 ± 0.1
72	7.7 ± 0.3	7.8 ± 0.1	8.1 ± 0.1	8.1 ± 0.1
96	7.7 ± 0.2	7.8 ± 0.1	8.1 ± 0.1	8.1 ± 0.1
120	7.8 ± 0.2	7.9 ± 0.1	8.1 ± 0.1	8.0 ± 0.2
144	7.7 ± 0.3	7.9 ± 0.1	8.1 ± 0.1	8.1 ± 0.1
168	7.6 ± 0.4	7.7 ± 0.2	8.1 ± 0.1	8.0 ± 0.1

Table B-2. Specific conductance (µS/cm; mean ± SD) measured over 168 hours, including influent (hour = 0), in the layered biofilm experiments for both substrates, treatments have been differentiated as control (no biofilm) and biofilm (all columns with biofilm, either covered or uncovered).

Time (hours)	Recycled glass		Mussel shell	
	Control	Biofilm	Control	Biofilm
0	26.2 ± 8.1	100.1 ± 29.4	26.2 ± 9.6	90.2 ± 48.9
24	66.1 ± 32.3	125.0 ± 13.4	378.5 ± 164.4	326.2 ± 84.7
48	73.4 ± 35.6	128.5 ± 15.4	436.5 ± 180.8	404.0 ± 129.1
72	77.3 ± 37.9	131.2 ± 16.1	474.7 ± 189.3	452.3 ± 151.0
96	84.9 ± 43.1	133.2 ± 17.1	508.5 ± 203.2	486.0 ± 171.8
120	88.1 ± 44.3	135.4 ± 17.1	535.5 ± 203.6	513.5 ± 183.4
144	90.7 ± 45.6	136.8 ± 17.7	564.4 ± 209.5	535.6 ± 191.9
168	92.6 ± 46.3	138.6 ± 16.4	583.4 ± 204.8	549.7 ± 193.1

Phase Two – Whole Column Biofilm Experiments

Table B-3. pH (mean ± SD) measured over 48 hours, including influent (hour = 0), in the biofilm experiments for both substrates and treatments, treatments are control (no biofilm) and biofilm.

Time (hours)	Recycled glass		Mussel shell	
	Control	Biofilm	Control	Biofilm
0	5.7	5.9 ± 0.1	5.8	5.7 ± 0.1
2	7.6	7.4 ± 1.3	6.6	9.6 ± 0.0
4	7.5	8.6 ± 0.2	7.0	9.8 ± 0.0
6	7.1	8.9 ± 0.1	7.1	9.8 ± 0.0
12	6.7	8.0 ± 0.3	7.6	9.6 ± 0.0
24	7.0	7.6 ± 0.2	7.7	9.2 ± 0.0
48	7.5	8.2 ± 0.1	7.7	9.1 ± 0.0

Table B-4. Specific conductance (µS/cm; mean ± SD) measured over 48 hours, including influent (hour = 0), in the biofilm experiments for both substrates and treatments, treatments are control (no biofilm) and biofilm.

Time (hours)	Recycled glass		Mussel shell	
	Control	Biofilm	Control	Biofilm
0	11.7	11.5 ± 0.0	11.5	12.2 ± 0.1
2	42.5	57.0 ± 6.5	137.6	234.9 ± 7.6
4	42.5	72.8 ± 8.3	203.0	350.3 ± 4.4
6	43.4	76.3 ± 8.1	277.5	360.0 ± 5.2
12	44.4	76.1 ± 7.5	339.7	356.7 ± 1.5
24	42.7	79.1 ± 7.9	392.7	394.7 ± 2.7
48	43.5	80.7 ± 7.5	450.3	484.1 ± 5.5

Gravel Experiments

Table B-5. pH and specific conductance (µS/cm; mean ± SD) results measured over 48 hours, including influent (hour = 0), in the gravel experiments.

Time (hours)	Gravel	
	pH	Specific conductance (µS/cm)
0	4.7 ± 0.1	18.9 ± 0.3
2	6.3 ± 0.0	19.8 ± 2.8
4	6.6 ± 0.1	22.6 ± 3.0
6	6.7 ± 0.0	25.2 ± 3.4
12	6.8 ± 0.1	29.1 ± 3.9
24	6.7 ± 0.1	35.4 ± 4.8
48	6.9 ± 0.1	46.5 ± 4.8

Batch Adsorption Experiments

Table B-6. pH (mean ± SD) results for recycled glass, mussel shell, and gravel from the batch adsorption experiments conducted over 48 hours, including influent (hour = 0).

Time (hours)	Recycled glass	Mussel shell	Gravel
0	5.8 ± 0.0	5.8 ± 0.0	5.8 ± 0.0
2	8.4 ± 0.0	8.4 ± 0.1	6.0 ± 0.0
4	8.1 ± 0.2	8.4 ± 0.1	7.4 ± 0.2
6	8.6 ± 0.0	7.9 ± 0.1	7.9 ± 0.1
12	8.7 ± 0.0	7.8 ± 0.1	6.6 ± 0.1
24	8.6 ± 0.0	7.6 ± 0.1	8.2 ± 0.1
48	8.4 ± 0.0	7.7 ± 0.1	8.0 ± 0.1

Appendix C – Effluent Metal Concentration Data

Gravel Experiments

Table C-1. Dissolved zinc, copper, and lead concentrations (µg/L; mean ± SD) in influent (hour = 0) and effluent for gravel experiments (n = 4).

Time (hours)	Gravel		
	Zinc	Copper	Lead
0	273.5 ± 1.0	112.0 ± 4.3	35.3 ± 0.5
2	78.3 ± 3.9	31.9 ± 6.6	9.0 ± 1.0
4	11.0 ± 2.3	7.2 ± 4.1	1.9 ± 0.5
6	3.4 ± 1.4	4.5 ± 0.5	0.8 ± 0.2
12	2.6 ± 1.9	4.6 ± 0.4	0.5 ± 0.2
24	2.6 ± 0.8	5.1 ± 0.6	0.3 ± 0.1
48	5.0 ± 4.9	6.8 ± 5.4	0.5 ± 0.6

Batch Adsorption Experiments

Table C-2. Dissolved zinc (Zn), copper (Cu), and lead (Pb) concentrations (µg/L; mean ± SD) in the influent (hour = 0) and effluent in the batch adsorption experiments, for the three substrates recycled glass, mussel shell, and gravel (n = 2 for each substrate).

Time (hours)	Recycled glass			Mussel shell			Gravel		
	Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu	Pb
0	289.2 ± 4.0	126.0 ± 1.6	37.3 ± 0.3	274.1 ± 19.3	119.6 ± 6.8	35.2 ± 1.8	268.3 ± 2.8	119.1 ± 2.6	35.3 ± 0.5
2	209.5 ± 20.8	65.6 ± 9.1	10.4 ± 2.2	46.6 ± 2.0	69.4 ± 14.5	1.4 ± 0.3	202.9 ± 2.2	60.1 ± 3.3	4.0 ± 0.6
4	69.7 ± 16.9	35.0 ± 3.4	6.9 ± 0.3	40.5 ± 1.0	61.0 ± 9.4	1.9 ± 0.2	102.5 ± 7.0	18.5 ± 1.0	1.1 ± 0.0
6	49.5 ± 19.9	27.9 ± 0.0	5.2 ± 0.2	35.2 ± 0.0	52.3 ± 11.1	1.5 ± 0.3	46.2 ± 4.8	6.6 ± .07	0.4 ± 0.0
12	17.1 ± 0.7	16.3 ±0.4	1.8 ± 0.1	12.1 ± 2.1	35.1 ± 11.7	0.6 ± 0.1	15.0 ± 1.1	3.4 ± 0.	0.2 ± 0.0
24	14.7 ± 1.0	14.8 ± 0.6	1.0 ± 0.0	8.4 ± 1.9	27.0 ± 10.0	0.2 ± 0.1	5.0 ± 0.2	2.7 ± 0.1	0.2 ± 0.0
48	22.6 ± 0.8	18.1 ± 1.1	0.9 ± 0.0	5.8 ± 1.1	20.0 ± 7.8	0.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	0.2 ± 0.0

Appendix D – Removal Efficiency Data

Phase One - Layered Biofilm Experiment

Table D-1. Dissolved zinc (Zn), copper (Cu), and lead (Pb) removal efficiency (%; mean ± SD) for the recycled glass and mussel shell substrates with all treatment conditions. Treatment conditions are no biofilm (substrate only, control; n = 4), biofilm grown exposed to light (uncovered biofilm; n = 4), and biofilm grown with no light exposure (covered biofilm; n = 4).

Time (hours)	Recycled glass								
	Control			Uncovered biofilm			Covered biofilm		
	Zn	**Cu**	**Pb**	**Zn**	**Cu**	**Pb**	**Zn**	**Cu**	**Pb**
24	94.7 ± 2.8	58.9 ± 6.4	95.6 ± 1.3	81.8 ± 3.7	57.6 ± 9.6	95.3 ± 2.1	73.9 ± 14.4	54.3 ± 10.5	95.3 ± 1.3
48	87.1 ± 2.8	60.1 ± 14.0	95.4 ± 1.8	86.2 ± 1.6	58.6 ± 6.0	95.9 ± 1.6	79.9 ± 8.7	48.3 ± 12.1	95.8 ± 0.8
72	80.7 ± 4.7	54.8 ± 18.1	95.0 ± 1.9	84.1 ± 2.9	58.0 ± 4.1	95.9 ± 1.8	81.4 ± 5.3	46.7 ± 12.3	94.9 ± 0.8
96	78.0 ± 1.7	54.3 ± 21.0	95.1 ± 2.4	83.4 ± 2.3	55.0 ± 6.5	95.8 ± 1.8	81.8 ± 3.7	46.3 ± 8.0	95.2 ± 1.2
120	76.9 ± 1.0	48.2 ± 25.0	93.9 ± 3.4	91.4 ± 0.7	52.8 ± 8.3	95.3 ± 2.0	84.7 ± 5.0	44.5 ± 4.3	94.4 ± 2.2
144	77.2 ± 0.9	48.7 ± 31.7	95.0 ± 2.7	88.4 ± 1.3	50.9 ± 7.8	94.9 ± 2.1	83.6 ± 3.3	43.0 ± 4.8	93.4 ± 2.8
168	77.4 ± 1.5	47.3 ± 30.1	93.6 ± 4.2	86.3 ± 1.1	56.7 ± 11.2	96.1 ± 2.4	85.4 ± 3.0	43.0 ± 7.1	92.4 ± 4.6
	Mussel shell								
24	96.8 ± 3.3	78.3 ± 28.7[1]	95.9 ± 3.6	98.1 ± 0.6	92.8 ± 3.3	97.7 ± 0.7	96.3 ± 3.4	92.3 ± 2.6	97.6 ± 0.9
48	98.2 ± 0.5	94.8 ± 2.7	98.2 ± 1.1	97.5 ± 1.0	92.5 ± 3.5	98.1 ± 1.2	97.6 ± 1.5	92.9 ± 4.0	99.0 ± 0.4
72	97.3 ± 1.8	93.5 ± 4.7	98.1 ± 1.7	82.2 ± 27.1[1]	92.4 ± 3.1	98.7 ± 0.7	98.7 ± 0.4	93.5 ± 3.5	99.0 ± 0.9
96	98.5 ± 0.3	94.7 ± 2.3	98.3 ± 0.9	88.5 ± 16.3	92.6 ± 2.7	99.3 ± 0.1	99.0 ± 0.1	93.8 ± 3.4	98.5 ± 1.3
120	97.7 ± 1.4	95.7 ± 1.2	95.7 ± 5.4	97.0 ± 1.7	92.4 ± 2.4	99.2 ± 0.2	99.0 ± 0.1	94.6 ± 2.7	99.2 ± .05
144	98.5 ± 0.6	95.8 ± 1.1	98.5 ± 1.3	97.2 ± 1.2	92.7 ± 1.8	99.0 ± 0.4	98.9 ± 0.3	94.8 ± 2.6	98.7 ± 1.2
168	98.2 ± 0.5	95.6 ± 1.3	98.7 ± 1.1	96.4 ± 1.9	93.0 ± 1.6	98.7 ± 0.8	98.9 ± 0.2	95.6 ± 1.4	98.7 ± 0.8

Note: 1. Outlier present in data

Phase Two - Whole Column Biofilm Experiments

Table D-2. Dissolved zinc (Zn), copper (Cu), and lead (Pb) removal efficiencies (%; mean ± SD) for the recycled glass and mussel shell substrates with treatment conditions. Treatment conditions are no biofilm (substrate only, control; n = 1), and biofilm (n = 3).

Time (hours)	Recycled glass					
	Control			Biofilm		
	Zn	**Cu**	**Pb**	**Zn**	**Cu**	**Pb**
2	32.5	50.5	59.8	40.5 ± 4.0	36.6 ± 3.5	44.0 ± 5.7
4	29.5	71.5	80.5	71.7 ± 2.8	60.2 ± 4.6	67.7 ± 5.0
6	35.1	77.3	8.6	85.2 ± 1.5	70.5 ± 3.8	80.5 ± 3.9
12	43.6	80.0	93.5	88.4 ± 3.6	71.4 ± 5.5	85.8 ± 4.4
24	52.2	82.7	97.3	82.3 ± 2.8	70.7 ± 7.1	87.9 ± 4.7
48	82.3	82.3	97.6	86.5 ± 4.8	67.9 ± 4.4	91.3 ± 2.8
	Mussel shell					
2	31.5	28.3	30.8	45.3 ± 1.4	32.0 ± 2.0	46.0 ± 3.1
4	50.1	44.2	49.6	80.3 ± 0.6	59.9 ± 3.8	77.3 ± 3.4
6	76.1	71.1	72.7	90.5 ± 0.4	74.9 ± 4.7	90.6 ± 0.2
12	97.1	94.0	94.3	98.4 ± 0.1	79.4 ± 4.7	95.1 ± 0.1
24	99.1	97.1	99.0	97.9 ± 0.2	81.2 ± 4.8	98.7 ± 0.4
48	98.9	97.0	99.0	97.2 ± 0.3	82.6 ± 8.6	97.9 ± 0.5

Gravel Experiments

Table D-3. Dissolved zinc (Zn), copper (Cu), and lead (Pb) removal efficiencies (%; mean ± SD) for gravel experiments (n = 4).

Time (hours)	Gravel		
	Zn	Cu	Pb
2	71.4 ± 2.2	71.5 ± 3.5	74.4 ± 3.1
4	85.2 ± 18.8[1]	93.5 ± 2.1	94.6 ± 1.5
6	98.8 ± 0.2	96.0 ± 1.3	97.7 ± 0.5
12	99.1 ± 0.1	95.9 ± 1.7	98.7 ± 0.6
24	99.1 ± 0.2	95.4 ± 0.7	99.2 ± 0.2
48	98.2 ± 1.9	93.9 ± 4.4	98.7 ± 1.7

Note: 1.Outlier present in data

Batch Adsorption Experiments

Table D-4. Dissolved zinc (Zn), copper (Cu), and lead (Pb) removal efficiencies (%; mean ± SD) for batch adsorption experiments, for the three substrates recycled glass, mussel shell, and gravel (n = 2 for each substrate).

Time (hours)	Recycled glass			Mussel shell			Gravel		
	Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu	Pb
2	27.4 ± 8.2	47.9 ± 7.9	71.9 ± 6.1	82.8 ± 2.0	41.1 ± 15.4	96.0 ± 1.0	24.4 ± 1.6	49.5 ± 1.7	88.6 ± 1.4
4	76.0 ± 5.5	72.2 ± 2.4	81.5 ± 0.1	85.2 ± 0.7	48.4 ± 10.7	94.6 ± 1.0	61.8 ± 2.2	84.5 ± 0.5	97.0 ± 0.0
6	75.9 ± 7.2	77.8 ± 0.3	86.1 ± 0.6	59.7 ± 28.3[1]	55.6 ± 11.8	95.8 ± 1.0	82.8 ± 2.0	94.5 ± 0.7	99.0 ± 0.0
12	94.1 ± 0.3	87.1 ± 0.5	95.1 ± 0.2	95.5 ± 1.1	70.0 ± 11.5	98.2 ± 0.5	94.4 ± 0.5	97.2 ± 0.3	99.4 ± 0.0
24	94.9 ± 0.3	88.2 ± 0.3	91.3 ± 0.1	96.9 ± 0.9	76.8 ± 9.7	99.4 ± 0.3	98.1 ± 0.1	97.8 ± 0.1	99.5 ± 0.0
48	92.2 ± 0.4	85.6 ± 1.0	97.5 ± 0.1	97.8 ± 0.5	82.9 ± 7.5	99.4 ± 0.2	99.2 ± 0.0	98.2 ± 0.0	99.6 ± 0.0

Note: 1. Outlier present in data